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The Journal of Laboratory and Clinical Medicine

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AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THE WARD BURDICK RESEARCH AWARD OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THE yeoman services of Dr. Ward Burdick in helping to organize the American Society of Clinical Pathologists, and as its Secretary to raise it to an enviable status, have led the members and the administrative officers to establish a memorial which will perpetuate his endeavors for the organization.

Dr. Ward Burdick assisted at the birth of the American Society of Clinical Pathologists, in St. Louis, on May 22, 1922, at a call that he sent out for a meeting which was destined to be the first successful national convention of clinical pathologists. On that occasion he was nominated temporary secretary and then became the permanent incumbent of this office when the Society was definitely formed. Dr. Burdick was eminently suited for this position. Of a calm and judicial temperament, painstaking and meticulous in the conduct of the affairs of the Society, imbued with a spirit of idealism which led him to devote all his spare hours in reflections how best to promote the progress of the organization, Dr. Burdick was a providential selection for the position in the early days of the organization when the crusading spirit was needed to gather forces and battle the evil spirit of commercialism that was beginning to infiltrate the clinical pathologists of the country.

He was never too busy to answer a call in behalf of the Society. Although his attendance at the meetings was fraught with peril to his health, he was present at the earlier conventions and took part in the deliberations even though he was suffering from a fever at the time.

It is only natural, therefore, that the Research Committee of the American Society of Clinical Pathologists for the year 1927-28 selected the idea of a medal as a fitting remembrance of one who had served the Society so conscientiously and as an example for the younger men to emulate the spirit of idealism, of progress, and of research, which was a dominant feature of

Ward Burdick's character. The Committee, consisting of Dr. H. J. Corper, Chairman, Dr. H. J. Nichols, Dr. H. S. Martland, Dr. Kenneth Lynch, Dr. Wm. C. MacCarty and Dr. W. G. Lyle, recommended to the Society that a fitting award be established to commemorate the name of Dr. Burdick, the prize to be in the form of a medal to be presented annually for some outstanding research project in the field of clinical pathology by a member of the Society. The present Research Committee appointed by Dr. Frank W. Hartman, President of the Society, consists of Dr. Alvin G. Foord, Chairman, Dr. Oscar B. Hunter, Dr. Stanley P. Reimann, Dr. W. G. Gamble, Jr., and Dr. A. S.

Giordano. It was the unanimous opinion of this committee that the award should consist of an appropriate gold medal bearing the profile likeness of Dr. Ward Burdick and the nature of the award on its face and on the reverse side the seal of the Society and the name of the recipient as well as the date of presentation.

The award is to be presented to the successful candidate by the President of the Society at the annual meeting on the evening of the banquet. The competition is now open to all the members of the American Society of Clinical Pathologists. All candidates for the award must present their theses at least two months prior to the annual meeting in order that they may receive the careful judgment of the Research Committee.

ETIOLOGY OF ACUTE LEUCEMIAS*

By A. S. Rubnitz, M.D., Omaha, Nebr.

IN THE last six years I have seen five cases of acute leucemia. Being greatly impressed with the similarity of the course and clinical picture of these cases and the still more striking meagerness of information regarding the etiology of the disease, it seemed worth while to attempt to systematize all the data on the etiology of leucemia.

I will not burden the audience with the detailed histories of these cases, as they were all typical cases of acute leucemia. Four of the patients were males of the following ages: six, fifteen, twenty, and sixty-six; one was a female, aged fifty-nine. Their leucocyte counts ran from 60,000 to 300,000; for a brief period only did the leucocyte count in one case (boy aged six) drop to 4,000; that happened shortly after extensive radiation. But soon after, the leucocyte count increased, reaching 100,000 before death. Clinically they all had, in a greater or lesser degree, the usual triad of symptoms: sepsis, necrotic inflammation of the oral cavity, and hemorrhages.

On first observation each one of them was apparently suffering from an acute infection. The questions to be answered were: Did the infection produce the leucemia, or was the leucemia produced by some other unknown factor, the infection being secondary to the lessened resistance of the tissues? I shall endeavor to answer these questions.

The literature on the subject of leucemia has been in the process of being written for the last eighty years. A more or less definite conception about this disease has been worked out and accepted by our textbooks; but even the well-accepted orthodox views are not final, and, I dare say, the near future will see the chapter on leucemias rewritten.

Our present day classification divides the leucemias (both acute and chronic) into the lymphatic or lymphogenic, and the myeloid or myelogenic.

The basis for this classification is the dualistic theory of Ehrlich. According to this theory all the leucocytes are divided into two main groups: the granulocytic and the nongranulocytic. The cells of the first group have their origin in the bone marrow, while the cells of the second group originate in the lymphoid tissue. A leucemia, therefore, is classed as myelogenic if the majority of the cells determining the diagnosis of leucemia are of the young granulocytic type. The same holds true for the lymphogenic variety.

This strict differentiation of leucemias may be fitting in the chronic types since the blood, as well as the clinical findings, and, to a certain extent, the clinical course, differ perceptibly in the two chronic varieties.

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

In acute leucemias, on the other hand, we do not have any appreciable differences either in the clinical or in the laboratory findings. Clinically all the acute leucemias are alike. They are all characterized by the same triad of symptoms, run the same clinical course, and have the same fatal termination.

The differentiation into acute lymphatic and acute myeloid leucemia is based on the supposedly different blood pictures. But does there exist such a sharp differentiation?

Some hematologists make matters even more complicated. They recognize a triple origin of leucocytes: (1) Bone marrow (polymorphonuclear cells and their progenitors, the myelocytes); (2) reticulo-endothelial system (monocytes); and (3) lymphoid tissue (lymphocytes and their progenitors, the lymphoblasts). Following this classification of the origin of leucocytes, they recognize a third variety of leucemia; namely, the monocytic leucemia. (Reschad and Schilling.[1])

It may not be out of place to mention here the views advanced by Pappenheim,[2] the chief exponent of the unitarian theory of the origin of leucocytes. According to him, all the leucocytes have their common origin in one cell, the large lymphocyte, and only through special differentiation do we get the two main classes of cells: granulocytic and agranulocytic.

When one studies mature cells he will be more inclined to accept a theory which supposes that the different leucocytes have different origins, but if one is given to the study of immature, embryonal cells, the unitarian theory of Pappenheim is more likely to appeal to him.

In acute leucemias we are always dealing with immature, embryonal cells. The presence of immature cells in the blood is perhaps the most essential requisite for the diagnosis of the disease.

The diagnosis of acute leucemia usually hangs upon a large mononuclear cells, which used to be considered in the old textbooks as a large lymphocyte. The cell usually has a vacuolated nucleus, with several nucleoli. There is marked unevenness in the density of the nucleus; granules are usually absent. In some cells the entire nucleus has a network of stringy matter. It is a cell one will always remember, once one has seen it. It is not a large lymphocyte, nor is it always a myeloblast. It is a very young form of a leucocyte, which is sometimes hard to place in the scale of classification. If you will consider it a very young lymphocyte or lymphoblast you may come just as near to the truth as considering it a young myelocyte or myeloblast.

Some hematologists consider the oxydase reaction the most deciding factor in differentiating the granulocytic cells (beginning with the myeloblast, down to the mature polymorph) from the agranulocytic cells (beginning with the lymphoblast, down to the mature small lymphocyte).

All the granulocytic cells, having their common origin in the bone marrow (according to accepted theories) give a positive oxydase reaction because they contain some ferment in them.

But the ferment content fluctuates with the maturity or immaturity of the cells. A very young myeloid cell, a myeloblast, or even a premyelocyte, may not give this reaction, so that only a positive oxydase reaction is of value, while a negative one does not mean much. In other words, we are often deal-

ing with a cell in acute leucemias which may be considered either a lymphoblast or myeloblast, depending on the morphologic interpretation of the observer. The type of leucemia, then, would be classed according to the interpretation.

Barchasch,[3] in summarizing his paper on leucemias, makes the following statement: "The protease or oxydase reaction is necessary in the differential diagnosis of lymphatic and myeloid leucemias." Yet in Case 3 of his report, he did not get a positive oxydase reaction; although he considers it as a case of myeloid leucemia. In discussing that case he remarks: "It is interesting that the myeloblasts of the given case, irrespective of the fact that they were so typical (myeloblasts), did not give the oxydase reaction; this can be explained on the basis of their belonging to the youngest forms (embryonal types)."

After all, a theory is in the majority of instances a logical explanation of observed or recorded facts. With all due respect to the authorities behind the dualistic or trialistic theories, the usual blood findings in acute leucemias warrant the acceptance of the unitarian theory of the origin of leucocytes.

Some other published data seem to substantiate the latter theory:

1. Pappenheim[2] calls attention to the following fact:

"In some lower invertebrates, which possess neither bone marrow nor lymph glands, we may still find both forms of cells in the blood, the granulocytes as well as the agranulocytes."

2. Ellermann and Bang's deductions (cited by Schmeisser[4]) on experimental leucemias in the fowl:

"Spontaneous and transmitted leucemias occur in two types: Myeloid and lymphatic: a myeloid leucemia may occur in the first generation, and a lymphatic in the next, or both types may occur in the same generation."

3. Parker and Rhoads,[5] in reporting their own as well as other observers' findings on incubated leucemic blood, record the following interesting observation:

"New embryonal cells (designated by the authors as 'X' and 'Y' cells) were developed from the blood of either cases: Myeloid or lymphatic leucemias. These cells vary from strongly oxydase-positive to oxydase-negative." Without going into further discussion on the origin of leucocytes, the logical deduction from the presented data would be: To Drop the Heretofore Accepted Subdivision of Acute Leucemias. We Should Consider a Case of Acute Leucemia as an Entity in Itself, Irrespective of the Possible Origin of the Cells, Myelo- or Lymphogenic.

Of my five cases the first could have been diagnosed as septicemia due to mouth infection, the second had "canker sores" in the mouth. The third did not have any apparent oral infection, at least at the time I examined him, but the fourth was a very outspoken case of gangrenous stomatitis. The fifth started with a "sore throat" and, when seen by me, had a continuous hemorrhagic condition of the oral mucous membrane.

Mouth infection, therefore, was apparent in 80 per cent of the cases. In all cases of acute leucemia reported in the literature, one will find mouth infec-

tion present in at least 60 per cent. One naturally will be led to think about the possibility of infection as the etiologic factor in this disease.

Ellerman and Bang (cited by Schmeisser) reported that they succeeded in transmitting leucemia from a spontaneous case in a fowl into other healthy birds. They used a filtrate of the spleen, liver, and lymph glands. They concluded that a cell-free filtrate is effective, and that, therefore, the cause must be an organized virus. In 1909 the same authors reported the transmission of the disease into the sixth generation with a Berkefeld filtrate. Their conclusions were: (a) Leucemia must be an infectious disease. (b) It is a disease due to a filtrable virus. Other investigators at that time were also successful in reproducing the disease with Ellerman and Bang's filtrate. Schriddle[6] questioned the deductions of Ellerman and Bang. He claimed that he produced changes similar to those of Ellerman and Bang by the injection of an extract of an entirely normal organ. In this country Schmeisser[4] reported his successful results in transmitting leucemia to the fowl using the methods of Ellermann and Bang. While all the above experiments point to infection as the etiologic factor in leucemia, yet no definite organism has been claimed by any observer to be the causative one. A variety of organisms have been found by different authors in association with this disease: typhoid-colon group, diphtheria bacillus and diphtheroids, Vincent's organisms, etc. It is generally accepted, though, that no known microorganism is responsible for the disease. Vincent's organisms are perhaps most commonly found in the oral cavities of acute leucemic patients, especially if there is any necrosis of the mucous membranes, but Vincent's organisms may be found in any condition of the mouth where sloughing is taking place. Therefore, while most of the leucemic patients suffer from a necrotic condition of the oral mucous membrane, yet we have very little evidence to suspect that leucemia is caused by any specific organism.

Let us accept for a time a different view: That leucemia is primarily a malignancy of the blood-forming tissues. We do get a hyperplasia of the splenic pulp, lymphoid tissue, bone marrow, and the reticulo-endothelial system throughout the body. Assuming that the hyperplasia and to some degree the metaplasia occurring in leucemia are the primary conditions caused by the same possible agents that are responsible for malignant growths in other tissues; assuming further that the malignant cells have multiplied in great enough numbers to poison the blood stream and develop a state of cachexia in the patient, we would be dealing then with a marked lowering of the resistance of the tissues to infection and almost any organism would act as a secondary invader.

The latter theory would not be very logical because we always find that the patient's health has been good until some infection developed which culminated in leucemia. The third possible theory would be that we are dealing with neither a malignancy of the blood-forming organs nor a specific infection, but rather with a special condition brought about or precipitated either by an infection or by an abnormal metabolic state.

Ward,[7, 8] for instance, in his revision of the literature found fifteen cases of leucemia during pregnancy and four in which the disease was recognized soon after parturition.

Accepting the last theory, we would define leucemia as *an unusual blood reaction to certain toxins;* the toxins may be produced by various agents, such as bacterial infections or metabolic derangements. The fact that we mostly find sloughing mucous membranes of the mouth associated with the disease would suggest the possibility that *necrotic mucous membranes may contain a toxic substance which is the causative agent.*

Schultz and Verse[9, 10] described a new disease which they named "agranulocytosis." This unusually fatal disease in many respects resembles acute leucemia. The total leucocyte count is generally very low; it may be below one thousand per cubic millimeter. The lymphocytes are relatively very high, while the granulocytes are pushed out of the picture until, before death, they disappear altogether from the peripheral circulation.

This disease should not be confused with leucemia, because we do not find there the immature, embryonal leucocytes. We are dealing rather with a condition in which the granulocytes are destroyed in the blood stream; when all of the granulocytes, or most of them, are gone, we have a lethal termination. The point of similarity between the two diseases is the necrosis of mucous membranes. The latter always occurs in agranulocytosis, and necrosis of the oral mucosa, vaginal mucosa, or even the intestinal mucosa has been described in connection with this disease.

It would seem logical to assume that the toxin in both agranulocytosis and acute leucemia is of the same quality, differing only in quantity.

In agranulocytosis the condition is much more active; the toxin is liberated in quantities sufficient to destroy the granulocytes in the blood stream. The hemopoietic system does not have any time to attempt any comeback, and the individual succumbs without a battle. We do not find, therefore, in such cases any perceptible histologic changes in the lymphoid tissues, bone marrow, spleen, etc.

In acute leucemia the quantity of the toxin is less. The granulocytes are destroyed in the blood stream, but not as fast as in the first condition. The blood-forming organs are stimulated to activity, with the result that numerous immature cells of every description are thrown out in the circulation. The condition lasts longer, although the outcome is usually the same. I say "usually the same," because we could picture to ourselves cases in which the toxin is liberated in insufficient quantities to kill the individual, and a balance of health might be reestablished.

It is generally accepted that any case that does not go on to a fatal termination should be excluded from the category of acute leucemias. This postulate may not always be true. Herz[11] reported a case and cited several others in which the blood picture was very suggestive of leucemia; clinically these cases suffered from an angina and necrotic condition of the mucous membranes of the palate and tonsils. The cases reported by Herz all recovered. His conclusion, therefore, is that leucemias are not all fatal.

The condition known as "infectious mononucleosis," which is most generally found in association with Vincent's angina, should also be included at the bottom of the list.

Speaking of septic infections secondary to necrotic inflammation of mucous membranes, Weiss[12] expresses the following views:

1. A septic infection may produce in some instances a simple monocytic reaction (monocytic angina, infectious mononucleosis).

2. A septic infection may exert its action on the blood-building tissues resulting in a hypogranulocytosis, without any multiplication of the general leucocyte count (symptom complex of Schultz), and lastly

3. A septic infection may produce a definite increase in the mononuclear leucocytes, with a hypogranulocytosis at the same time (acute leucemia).

All the three kinds of reactions have one thing in common, namely, necrotic inflammation of mucous membranes.

His deductions are that the hypogranulocytosis or agranulocytosis is not the cause of the necrotic inflammation of the mucous membranes, but both are disease symptoms, and the necrosis of the mucous membranes and the blood findings are caused by the septic infection.

But Weiss does not attempt to explain why the septic infection produces such a peculiar reaction in this type of cases. His deductions are not very clear. The point to be emphasized is that in septic infection of mucous membranes we get this peculiar reaction of the body which brings about the above-described clinical entities. Since we cannot ascribe these conditions to any specific infection, we conclude, therefore, that the cause must lie in the toxic substances liberated by necrotic mucous membranes. The toxin is very likely a definite chemical entity, a leucocytotoxin; it is lytic to granulocytic cells. The developing clinical superstructure depends on the quantity and rapidity of liberation of this particular toxin.

Agranulocytosis, acute leucemia, or infectious mononucleosis may all be produced by the same leucocytotoxin.

The different modifications and fluctuations of the number of leucocytes in leucemias depends very likely on the amount of toxin circulating in the blood stream.

Szillard[13] (cited by Barchasch) attempted to explain the fluctuation of the number of leucocytes in leucemic and pseudoleucemic bloods. He incubated a known quantity of the patient's leucocytes in the patient's serum; he then counted them after twenty-four hours' incubation. He found that in cases of subleucemic condition (where the number of leucocytes was low) the resistance of the leucocytes was low, while in cases where the number of leucocytes was high, the resistance of the leucocytes was much greater. His conclusions were that leucemic, subleucemic, or aleucemic conditions depended on the resistance of the leucocytes.

He failed, however, to take into consideration another possible factor, namely, that the cells were incubated in the patient's serum. The serum could carry in it a leucocytolytic substance which was responsible for the variation in the resistance of the leucocytes.

SUMMARY

1. There is no logical reason for the division of acute leucemias into lympho- and myelogenic, because

a. Clinically they represent one and the same entity.

b. Microscopically we cannot always draw a line between lymphogenic and myelogenic cells. The oxydase reaction is only of value if positive.

2. Acute leucemias, while always associated with infection, are not caused by any specific microorganisms.

3. Necrotic inflammation of mucous membranes is usually associated with acute leucemias as well as with the condition known as "agranulocytosis."

4. Some obscure toxin liberated by necrotic membranes, by nature a leucocytolysin, is very likey responsible for both acute leucemia and agranulocytosis.

5. Acute leucemia and agranulocytosis may be caused by a toxin of the same quality; the difference in the body reaction is quite likely due to quantitative variations in toxins produced and liberated.

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RESULTS IN VARIOUS DISEASES FROM ELIMINATION OF FOCI OF INFECTION AND USE OF VACCINES PREPARED FROM STREPTOCOCCI HAVING ELECTIVE LOCALIZING POWER*

By Edward C. Rosenow, M.D., and Allen C. Nickel, M.D.
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THE results from a long series of experiments on focal infection and elective localization reported during the last fourteen years have shown that: (1) foci of infection, wherever found, commonly harbor the causative organism of various systemic diseases, (2) they afford favorable conditions for the entrance of bacteria and their toxic products where bacteria tend to acquire and maintain relatively high or peculiar invasive powers, and (3) the situation, aside from predisposing factors and hence to a large degree the character of the systemic disease, depends on the specific or elective localizing power of the infecting organisms, in most instances streptococci. This statement, based on firsthand knowledge of clinical conditions, the bacteriology involved and animal experiments, is considered a good working hypothesis in clarifying the confusion which still exists in the minds of many regarding the application of the underlying principles involved. Even Holman, after his critical review of the work on focal infection and elective localization, in which he conceded the great importance of the former, yet regards proof of the latter as insufficient, stated: "However, what Rosenow and his followers particularly showed and what all other investigators of the problem have definitely demonstrated is that streptococci do localize in various organs and tissues and can produce lesions at least sufficiently suggestive of those found in man so that their potential danger in infected foci cannot be neglected."

The large number of instances reported in the literature during this period (fourteen years) in which causal relationship between focus and systemic disease is indicated by the clinical history and improvement or disappearance of the systemic condition following removal of foci of infection such as occur commonly in tonsils, teeth, sinuses, intestinal tract, prostate and cervix would alone be convincing if it were not that a considerable number of patients fail to improve following removal of one focus or more. Such failure, however, should not be interpreted as nullifying the principle involved, for there are good reasons why clinical improvement may not follow the removal of one or more foci of infection. The disease may have existed for so long a time as to produce irreparable harm or it may have become so thoroughly established as to continue independent of a primary focus. The particular focus or foci removed may not have had etiologic significance at the time of removal, or even before, or as in the case of dental or tonsillar foci, the opera-

*Read before the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 9, 1928.

From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minn.

tive procedure may have been inadequate. The responsible focus may have been overlooked because so often symptomless. Not all systemic diseases are expressions of focal infection. Clinical improvement occurred with such regularity and was so marked, however, following removal of foci of infection in those cases in which the results in animals in our experiments had demonstrated the presence of the causative organism as to leave no doubt of the great value of this procedure in suitable cases. We believed much valuable information might be obtained if clinical manifestations were studied in conjunction with first-hand knowledge of the bacteriology and the results obtained in animals following injection of cultures freshly isolated from suspected atria of infection or tissues involved. Moreover, since the injection into animals served so often to separate saprophytic from pathogenic or causative strains commonly indistinguishable by cultural means, and since in a few cases marked improvement was noted following the use of vaccines prepared from the bacteria isolated from the systemic lesions of injected animals, we determined also to study the therapeutic effect of these truly autogenous vaccines. The excellent clinical and laboratory facilities afforded at The Mayo Clinic and The Mayo Foundation are particularly well adapted for such study.

Most of the patients included in this report were seen by one or both of us with various members of the clinical staff during the last six years. Many of the patients presented puzzling diagnostic problems, particularly regarding the likelihood of etiologic relationship to one or more foci, their situation and whether or not to remove suspected foci of infection. The decision regarding any one or all of these points was not always made on clinical considerations alone but on these and the results of the animal experiments. Thus, sometimes questionable foci were or were not removed, depending on whether or not the animals injected with material or cultures from such foci developed lesions in structures corresponding to those affected in the patient. On the basis of previous studies clinicians felt that the results in animals might throw light on the differential diagnosis in certain cases. The great majority of patients had previously sought in vain for relief from a chronic, often progressive condition, and therefore if what was advised to be done resulted in amelioration or relief of symptoms we could be fairly certain that this was not accidental but was due to the treatment instituted.

METHODS OF STUDY

The cultural methods employed were similar to those reported by either of us at various times. The condition of the patient at the time of study was also considered. A higher incidence of localization and more marked lesions in animals were obtained if cultures were secured at the time of acute systemic manifestations or during exacerbations of symptoms in chronic conditions than those obtained during quiescent intervals. Great care was exercised in the collection of material for study both as regards the clinical observations and the prevention of contamination by extraneous organisms.

If animals were injected intracerebrally in case of disease of the central nervous system and if they were injected intravenously in case of other diseases, and symptoms or lesions developed similar to those in the patient from

whom the culture was obtained, the causative organism, nearly always a green-producing streptococcus, was regularly recovered in pure culture or with only scattered colonies of other bacteria, from the injected animal. The vaccine used for treatment was usually prepared from the streptococcus thus isolated and in most instances represented the first, more rarely the second, and seldom a higher subculture. In some cases the vaccine administered was prepared from heterologous strains of the streptococcus which had produced symptoms in animals simulating those of the patient. In other cases, such as furunculosis it was prepared directly from the freshly isolated bacteria without injection of animals.

Various methods have been used in preparing the vaccine. Many consisted of the killed twenty-four-hour culture in glucose-brain broth or glucose

TABLE I

PERCENTAGE INCIDENCE OF ASSOCIATED INFECTIONS*

DISEASE	CASES	MALE	FEMALE	TONSILLITIS	INFLUENZA		FOCI OF INFECTION IN:						
					REMOTE	IMMEDIATE	TONSILS OR TONSILLAR TAGS	TEETH (PYORRHEA)	SINUSES	PROSTATE OR SEMINAL VESICLES	CERVIX	PULPLESS TEETH	
												CASES	POSITIVE (PER CENT)
Encephalitis	205	148	57	46	37	32	70	56	2	16	2	102	87
Arthritis	109	67	43	63	40	3	51	69	6	94	53	74	61
Spasmodic torticollis	32	23	9	53	41	6	60	75	3	57	11	17	76
Multiple sclerosis	27	19	8	33	41	15	78	67		37		19	89
Chronic poliomyelitis	17	12	5	47	30	18	65	71		25		13	92
Neuritis and myositis	17	10	7	71	35	24	41	71	24	80	57	12	42
Gastroduodenal ulcer	27	25	2	33	37		74	74		72	100	21	52
Lesions of eye	19	9	10	58	42	5	53	53		100	60	10	50
Lesions of skin	23	18	5	35	26	9	74	56	9	72		13	77
Prostatitis	24	24		42	54		63	54	4	100		13	69

*In computing the percentages in Tables I and II any fraction less than one-half is dropped; if the fraction is one-half or more the next higher figure is given.

broth, and a few of the killed cultures in pancreatic digest broth. About two-thirds consisted of the sodium chloride suspension of the density of the broth cultures made directly from the unwashed sedimented bacteria or at various intervals from those preserved in dense suspension in glycerol, two parts, and saturated sodium chloride solution, one part. One cubic centimeter of these dense suspensions was made to represent the sediment after centrifugalization of the growth from approximately 120 c.c. of the eighteen to twenty-four-hour broth culture. In these, viability of some of the bacteria was preserved for many weeks, and specific immunologic properties, with few exceptions, were preserved almost indefinitely when the bacteria were kept in the ice box. The bacteria were killed by adding 0.5 per cent phenol or 0.3 per cent tricresol to the broth culture or the diluted sodium chloride solution suspension of the density of the broth culture, and if not sterile in twenty-four hours, by heating once, or at the most twice, at 60° C. for thirty minutes.

The vaccines were given subcutaneously in increasing doses once or twice a week beginning with 0.1 c.c. or about 200,000,000 dead bacteria and increasing gradually by 0.1 c.c. until 1 c.c. or about 2,000,000,000 were given provided the reaction in the patient remained minimal and the results obtained were seemingly favorable. The dosage was almost never increased above 1 c.c. We soon learned to avoid marked local or constitutional reaction for the patient's symptoms were nearly always worse in consequence. Hence, the patient's condition and reaction rather than the size of dose were used as the main guide to dosage. The succeeding dose was reduced and the interval made correspondingly longer if the one previously caused a marked local or constitutional reaction due to high toxicity of the vaccine, or to what appeared clearly to be an inherent or acquired hypersensitiveness in the patient.

RESULTS

The percentage incidence of associated infections, particularly focal infection, in the wide range of diseases studied (Table 1), was very high. The causative streptococcus often was isolated not only from badly infected tonsils but from tonsils that appeared small and normal but which on removal were large and sometimes contained abscesses, from unsuspected remnants of tonsils, and from symptomless roentgenographically positive and negative pulpless teeth, root tips and residual areas which had not been considered previously or which were thought to be harmless. A study of the figures expressing the incidence of remote and immediately previous attacks of influenza shows that the incidence of the former, while relatively high in all, does not vary greatly in the different groups, whereas the latter appears to have had etiologic significance in diseases of the nervous system.

The high incidence of cervicitis in patients with neuritis and myositis (57 per cent), lesions of the eye (60 per cent), and arthritis (53 per cent), is believed of significance because wholly absent or present in only a few cases of the other groups studied.

The incidence of prostatitis associated with or without seminal vesiculitis varied from 16 per cent to 100 per cent in the different groups. The incidence of 16 per cent in the encephalitis group is certainly too low because in a large number of the earlier cases special examination was made only in those cases in which there were suggestive symptoms of prostatitis.

The interval that elapsed from the time of our study and the reply to the questionnaire varied from one to five years. Not all of the patients seen were advised to have foci removed or to take the vaccine despite the fact that in the last few years a fair number came to The Mayo Clinic because some one of their acquaintance had been benefited by this treatment. Some could not stay to have the foci removed and for one reason or another failed to have it done after returning home. Others that had one focus or all foci removed, failed to reply to the questionnaire, some were lost trace of, a few died, and in others the information obtained was too meager to have statistical value. Satisfactory data were received concerning 358 (72 per cent) of the 500 patients from the attending physician, from reexamination, from personal letter, or from the questionnaire sent to the patient.

In Table II we have summarized the results obtained following removal of foci of infection and use of vaccine. In most cases the symptoms had existed for a long time and were gradually increasing, despite everything that had been tried previously. Striking examples of improvement following removal of foci and use of vaccine occurred in each of the groups studied, especially where all foci were dealt with. In order to conserve space details of cases will be reported later, elsewhere. Of the thirty-nine patients in whom not all foci were removed and who were vaccinated, 26 per cent improved, 46 per cent remained the same and 28 per cent grew worse. Of the twenty-eight patients in whom not all foci were removed and who were not vaccinated, only 21 per cent improved, 32 per cent remained the same and 47 per cent grew worse. In contrast, of the 216 patients in whom all foci were removed and who received the vaccine, 61 per cent improved, 17 per cent remained the same, and 22 per cent grew worse. Of the seventy-five patients in whom all foci were removed but who were not vaccinated, 54 per cent improved, 27 per cent remained the same, and 19 per cent grew worse. Altogether 358 cases were studied. Of this group 149 were encephalitis, mainly of a chronic progressive type, and in only eighteen had symptoms lasted five months or less. There were eighty-one cases of arthritis, most of which were of the progressive deformans type. All of the twenty-five cases of spasmodic torticollis were of the idiopathic type without any antecedent history of encephalitis. In sixteen of the seventeen cases of multiple sclerosis symptoms had been present for seven months or more and in eleven of these the symptoms were progressive. There were eleven cases of chronic poliomyelitis and thirteen cases of neuritis or neuro-myositis, in all of which the symptoms had lasted more than six months. Of the twenty cases of ulcer, one was gastric ulcer, nineteen were duodenal ulcer, in six of which gastrojejunal ulcer had developed following gastroenterostomy. Of thirteen cases of lesions of the eyes, seven lesions were progressive, two were stationary, and four were in a period of exacerbation. In all of the fifteen cases of lesions of the skin the symptoms had existed six months or longer. There were fourteen cases of prostatitis. In two cases the condition was acute. In twelve it was chronic.

TABLE II

RESULTS FROM REMOVAL OF FOCI OF INFECTION AND USE OF VACCINES PREPARED FROM STREPTOCOCCI THAT HAD ELECTIVE LOCALIZING POWER

VACCINE	ALL FOCI NOT REMOVED				ALL FOCI REMOVED			
	CASES	IMPROVED PER CENT	SAME, PER CENT	WORSE, PER CENT	CASES	IMPROVED PER CENT	SAME, PER CENT	WORSE, PER CENT
Used	39	26	46	28	216	61	17	22
Not used	28	21	32	47	75	54	27	19

DISCUSSION

The incidence of systemic or metastatic disease in the different groups, contrary to what is generally believed by clinicians, was comparatively low following acute infections of the respiratory tract, such as tonsillitis and influenza, and when it did occur there was usually an interval of one to four weeks after the acute attack subsided, a point emphasized in appendicitis by

Evans. This is what one should expect for the organisms, usually streptococci, the cause of the acute attacks. They are of high virulence and of high antigenic power, whereas the strains isolated from more or less symptomless foci or from involved tissues, such as of joints, ulcer of the stomach, and in cases of cholecystitis, and appendicitis, are of low general virulence, of lower antigenic power but of high elective localizing power. Hence, the consideration of focal infection in the etiology of disease must go much further than is indicated by the clinical history of acute manifestations in structures that have been shown to be the seat of low-grade infections although often symptomless yet teeming with living streptococci that frequently possess elective localizing power.

Owing to the fact that so many of the patients seemed to harbor more than one source of infection, it was impossible to determine accurately the relative importance of each. Judging from clinical evidence, however, and the results of cultures and animal experiments, it appears to us that localized infection in or around the following tissues, predisposes to disease in remote parts of the body in the order named: tonsils, teeth, especially pulpless teeth, paranasal sinuses, prostate, cervix, and intestinal tract. Considering the fact that in most instances the disease had existed for a long time in the different groups, that it was often progressive and far advanced, and that the general measures advised were not much different from those which had been employed previously, there would seem to be no doubt but that the relief obtained (Table II) was attributable chiefly to the elimination of foci of infection because it was so marked and in proportion to the thoroughness of removal and, to a lesser degree, to the use of the autogenous vaccine. Improvement occurred with striking regularity when foci were removed in which the causative streptococcus was demonstrated. The fact that exacerbations occurred following removal of foci in some cases also indicates causal relationship and perhaps hypersensitiveness of the affected tissues.

In justice to the patients it was of course impossible to limit the treatment to removal of foci and the use of the vaccine, to the exclusion of various other therapeutic measures. Hence, it was not always possible to determine precisely the benefits that were derived from the removal of foci with or without the use of the vaccine and those from the other measures employed.

As has been indicated, the patients studied represent in one respect or another unusually difficult cases in each of the groups of diseases and therefore are not comparable with the patients seen as a routine by clinicians. Many of them had not been relieved previously by the use of the usual therapeutic measures, such as frequent feedings and alkalies in cases of ulcer, local applications in diseases of the skin, and physiotherapy, diathermy (usually insufficiently and intermittently used), diet and orthopedic measures in arthritis, but responded, often in a striking manner, when these measures were supplemented by the removal of foci and the use of specific vaccines.

The results especially as regards the importance of focal infection in the cases of ulcer are in general agreement with the clinical observations of Frick, Judd, Eusterman and Rivers, and as regards elective localization and vaccine with the experimental studies of Nickel and Hufford, Nakamura, and Meisser

in ulcer of the stomach and duodenum. In the cases of arthritis the results are in agreement with those of Billings, Nakamura, Moench, and Nickel in chronic arthritis, of Starling in rheumatic infection, and of Rosenow in rheumatic fever. In diseases of the eye the results are in agreement with those of Benedict, Irons and Brown, Haden, Benedict, Von Lackum and Nickel, and Ravdin. In diseases of the skin the results are in agreement with those of Ravitch and Ravitch and Steinberg, and in prostatitis with those of Von Lackum, and Von Lackum and Holloway. After a preliminary survey of cases of encephalitis, Doyle concluded that his information was not sufficient to prove the value of vaccination. Ziegler, in a recent compilation of cases of encephalitis, found that the incidence of improvement in symptoms as measured by the patient's ability to work was not greater in the cases in which vaccine was given than in those in which it was not given. The mortality, however, among the sixty-nine patients vaccinated was only 4.3 per cent in contrast to 24 per cent of the 125 control patients in this group who were not vaccinated. Ziegler's cases in which vaccine was given, although included in our study, were not subdivided as regards partial or complete removal of foci of infection and therefore his results are not entirely comparable with ours. As would be expected, the results in the encephilitis group, considering the average long duration and the organic changes which no doubt had occurred, were the least favorable of any.

According to the results summarized in Table II, the benefit derived from the removal of foci of infection was increased by only about 7 per cent in the cases in which vaccine was given as compared with cases in which it was not given. This figure is lower than a study of the reports of the cases would indicate, since frequently patients felt better during the period of vaccination, were worse when the vaccine was discontinued, and again improved on resumption of the vaccine. This occurred repeatedly in some cases over a period of several years and certainly was not psychic in origin. We believe that this low average is due in large part to the fact that cases were included in which the chances for relief were well-nigh out of the question, as well as cases in which the attending physician followed the schedule of dosage in cubic centimeters instead of the minimal reaction of the patient. This sometimes led to inadvertent overdosage resulting in nonimprovement or temporary exacerbation of symptoms. Eliminating these cases, the results from vaccine were more favorable than is indicated in Table II. None of the patients, following injection of more than 4200 properly gauged doses of vaccine was made worse permanently. The results from the use of "stock vaccine" made from heterologous strains but true to type and preserved in dense suspensions of glycerol and saturated sodium chloride solution sometimes for many months were seemingly as good as those from freshly prepared autogenous vaccines. Hence, the practicability of the method is assured. The favorable clinical results from the use of these autogenous vaccines are in accord with the fact that specific protection has been obtained in animals against streptococci from ulcer and encephalitis, respectively, through immunization with the corresponding vaccines. Since the streptococci having elective localizing power belong to the viridans type in each of the diseases studied (the type con-

ceded by Holman to possess high invasive power and to occur with great regularity in suspected foci and which in other diseases localize in the tissues of animals and bring about lesions comparable to those in man), since improvement occurred in suitable cases following removal of foci and the use of the specific vaccine made from these streptococci, and since streptococci were demonstrable in the lesions in some of the diseases, the conclusion that the streptococci isolated had etiologic relationship seems warranted. Hence, aside from the benefit afforded patients by the removal of foci of infection and much corroborative evidence of previous work, we regard the ability to isolate the causative organism (usually streptococci from various infection atria in patients suffering from diverse diseases) by the methods outlined as of prime importance, for it makes possible, what otherwise would not be possible in many of the diseases studied, attempts at specific prevention and treatment that may reasonably be expected in the near future to do far more good than the heat-killed vaccine which we have used. Since streptococci appear to be a cause of so many diseases, since immunity is of short duration, and since mechanical factors play so large a part in maintaining infection in structures so commonly the seat of foci, even after prolonged use of specific vaccines, mechanical correction or removal, so far as possible, will probably always be necessary even if highly effective specific or other remedial agents are discovered. Recurrence of the previous condition or new localizations are prone to occur unless the predisposing cause, the focus, is eliminated. Likewise, operation will probably always be indicated in many of the chronic systemic lesions, such as chronic indurated ulcer, cholecystitis with gallstones, and appendicitis with fecal stones or constricted lumen from previous attacks.

Considerable evidence has been obtained in this study which suggests that tissues in the cases of various chronic diseases, particularly the diseased tissues, become hypersensitive to the infecting organisms and their toxic products, as indicated by exacerbations which are particularly prone to occur following too strenuous or incomplete removal of foci of infection, and by the reactions which sometimes follow injection of autogenous vaccines, a point stressed by Kolmer. If the dosage of vaccine is gauged so that the local and constitutional reactions are relatively slight the tolerance may often be greatly increased, even tenfold, and the patient improves, a result in accord with that of Wherry and others in bronchial asthma. If the dosage is too large tolerance may not increase but actually diminish, reactions may become progressively more marked with each succeeding injection and the patient's condition grow worse. This narrow limit of effective dosage determined more by the reaction in the patient than by the number of bacteria and the use, without bacteriologic diagnosis, of stock vaccines made from bacteria long cultivated in the laboratory have, we believe, contributed much to the general disrepute into which vaccine therapy has unfortunately fallen.

CONCLUSIONS

Foci of infection and the elective or specific localizing power of green-producing streptococci contained therein appear to be of etiologic significance in the diseases studied.

Either the cautious removal of foci of infection or the use of vaccines prepared from streptococci having elective localizing power, or both, are rational forms of treatment that have been proved of value in our experience and are worthy of further trial. The application of such treatment soon after the onset of disease or even before as a preventive measure, is especially indicated.

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PURPURA HEMORRHAGICA: REPORT OF THREE CASES*†

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VERY little is known of the clinical pathology of the purpuric diseases aside from a more or less constant low blood platelet count, prolonged bleeding time, deficient contractility of the clot without any marked delay in the coagulation time.

There are many classifications of purpura, based largely upon its clinical manifestations and the complicating sequellae or the concomitant diseases with which it is associated. Greene[1] states that it exhibits but two constant and invariable symptoms, imperfect thrombus formation and subcutaneous hemorrhages. He groups the different clinical forms as follows:

1. Complicating purpuras
2. Purpuras with arthritic manifestations
3. Simple purpura
4. Purpura hemorrhagica
5. Purpura fulminans.

The first includes those seen occurring in acute infectious processes and chronic diseases with cachexia; the second is associated with joint involvement and is usually subdivided into two types: Peliosis rheumatica or Schonlein's disease with the manifestation of moderate symptoms; and Henoch's purpura with severe symptoms, visceral crises, and acute nephritis.

The simple purpura, purpura hemorrhagica and fulminating purpura seem to be very similar idiopathic exhibitions of the same condition, differing largely in the degree of hemorrhage, without associated joint lesions or other complicating or predisposing manifestations. These types are most often designated as the idiopathic purpuras.

The three cases which we have to report appear to belong to this group, being neither typically simple nor extremely severe, but appear to be a moderate form of hemorrhagic purpura (*morbus Werlhofi*) with hemorrhages into the skin and from mucous membranes at varying periods; not alarming or intractible, associated with irregular febrile attacks and no marked constitutional symptoms, except those from hemorrhage and anemia.

CASE REPORTS

Case 1.—Miss A. B., was a young girl of American parents with irrelevant family history except that her father died of pulmonary tuberculosis, She had the usual diseases of childhood with good recovery and no sequelae. Her general health had been good and her habits normal. In the spring of 1924 when she began to menstruate it was noted that

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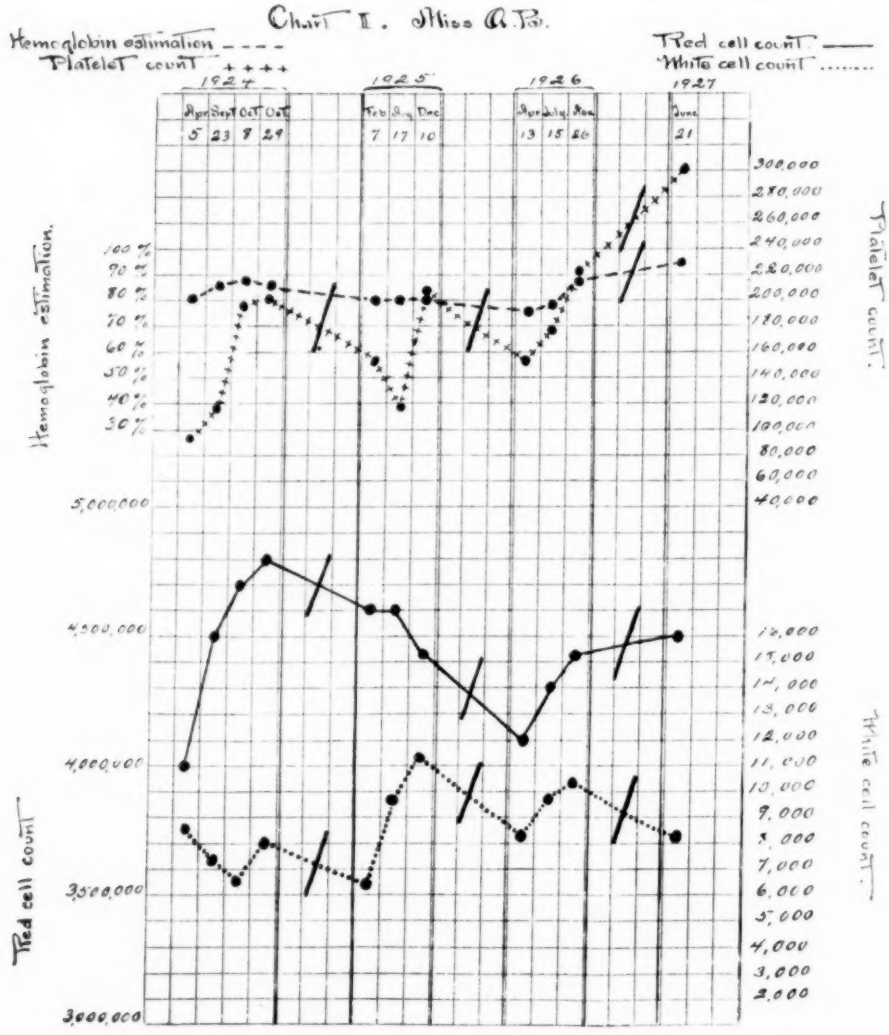
purpuric spots appeared, most numerous on her left arm and also to some extent on her thighs, chest and back; this was associated with some bleeding from her gums and rather profuse menstruation.

When first seen by us, April 5, 1924, her condition appeared to be that of a simple purpura. She showed a mild degree of anemia (see Table I) with no significant changes in her blood picture other than a reduced platelet count of 92,000. Her coagulation time was normal and bleeding time prolonged to seventeen minutes. Her Wassermann test was negative and her physical condition revealed nothing of a specific or significant character



except a palpable spleen. She was given three transfusions of 500 c.c. each of whole uncitrated blood, following which she showed marked improvement during the summer and early fall. She was not seen again until February, 1925, when she had a relapse, with irregular response to treatment until December of that year, when she again improved markedly. Following this she was not seen until April, 1926, when she had another relapse, but responded well to transfusion and apparently made a complete recovery.

TABLE I

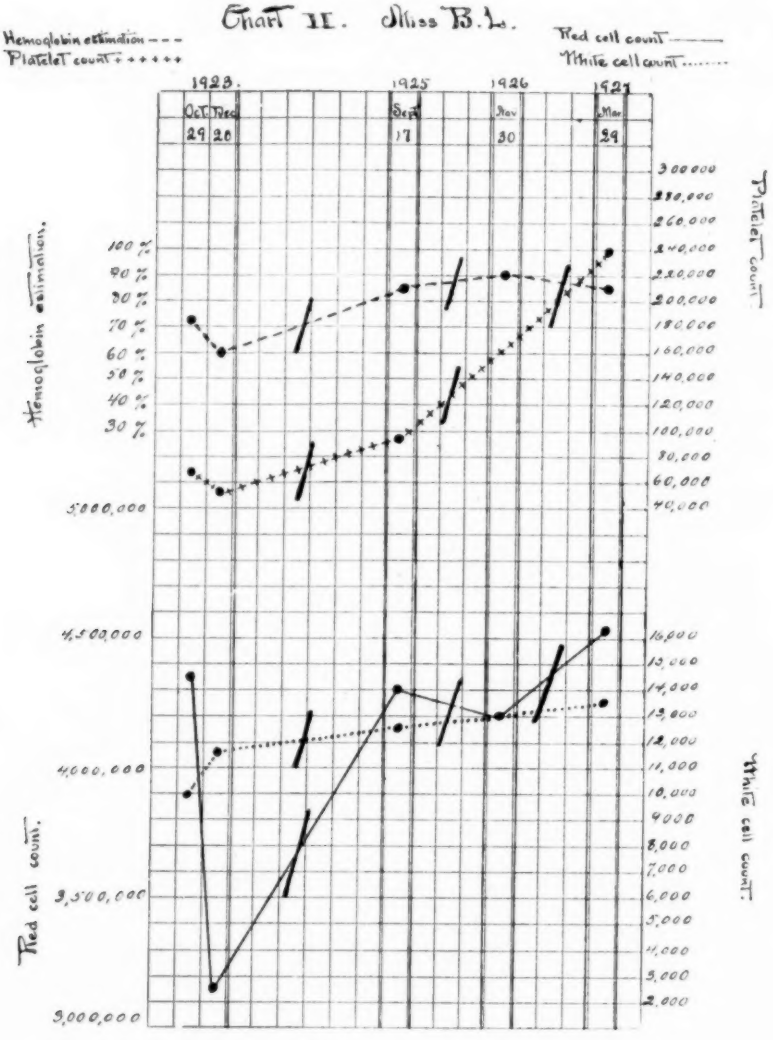
RECORD OF BLOOD EXAMINATIONS

CASE	DATE	HEMOGLOBIN	ERYTHROCYTES	PLATELETS	LEUCOCYTES	DIFFERENTIAL COUNT PER CENT				
						POLYMORPHONUCLEAR NEUTROPHILES	LYMPHOCYTES	LARGE MONONUCLEAR	EOSINOPHILES	BASOPHILES
1	4/ 5/24	80	4,000,000	92,000	8,600	71	23	2	4	
	9/23/24	86	4,500,000	110,000	7,400	73	18	5	4	
	10/ 8/24	88	4,700,000	198,000	6,600	58	24	8	10	
	10/29/24	86	4,800,000	200,000	8,000	53	40	5	2	
	2/ 7/25	80	4,600,000	152,000	6,400	60	34	1	3	
	8/17/25	80	4,600,000	118,000	9,600	71	23	5	1	
	12/10/25	80	4,450,000	210,000	11,400	86	11	1	2	
	4/13/26	76	4,000,000	152,000	8,200	73	23	2	2	
	7/15/26	78	4,200,000	175,000	9,800	78	20	1	1	
	11/26/26	88	4,420,000	220,000	10,400	80	15	1	3	1
	6/21/27	85	4,500,000	300,000	8,300	75	20	1	4	
2	10/29/23	72	4,400,000	68,000	10,000	77	20	2	1	
	12/20/23	60	3,100,000	50,000	11,600	75	17	4	3	1
	9/17/25	85	4,300,000	94,000	12,600	72	20	3	4	1
	11/30/26	90	4,200,000	–	13,000	55	35	3	5	2
	3/29/27	85	4,520,000	235,000	13,600	78	18	1	2	1
3	9/13/27	40	2,430,000	31,500	14,900	64	30	4	2	
	9/19/27	47	2,690,000	73,200	9,500	68	24	3	3	2
	9/24/27	49	2,890,000	138,300	10,100	70	20	5	4	1
	10/ 1/27	54	3,000,000	208,000	12,200	–	–	–	–	–
	12/29/27	60	3,830,000	460,000	10,100	75	20	2	2	1
	2/ 1/28	70	4,180,000	380,000	9,200	67	28	3	2	

CASE 2.—Miss B. L., was a Jewish girl, twelve years of age; without significant family history, except that her father died of pulmonary hemorrhage, of unknown cause. She had had measles, chicken-pox, whooping cough, and mumps, with good recovery and no sequelae. Her general health had been good and her habits normal, except for chronic constipation, which was difficult to relieve. During the summer of 1923, she developed purpuric spots scattered more or less over her entire body, bleeding from her gums and blood in the stool, with subsequent anemia.

When first seen by us on October 29, 1923, her condition was said to have improved. At this time she was moderately anemic; her blood picture was not especially significant, except for the blood platelet count of 68,000 and a prolonged bleeding time of twenty minutes. Her Wassermann test was negative and her physical condition was that of a poorly nourished anemic child, with numerous scattered purpuric spots over her body, and spongy, bleeding gums. A diagnosis of simple purpura was made. She was not seen again until December of the same year when she had a rather severe relapse, at which time her platelet count had dropped to 50,000 and her erythrocytes to 3,100,000 (see Table). At this time she had a palpable spleen. A diagnosis of purpura hemorrhagica was made and transfusion with splenectomy was advised. This patient was not seen again until September, 1925, shortly after a splenectomy in another city, details concerning which are lacking. Her condition was considerably improved; all bleeding had ceased and she had begun to put on weight. A history was obtained to the effect that she had begun to menstruate during 1924, and had had a very severe relapse from which she nearly died, during which time she was transfused. Subsequently her spleen was removed. She thereafter continued to improve until last seen in March, 1927, when her condition appeared to be excellent, except for the continued persistently high leucocyte count.

CASE 3.—Mrs. H. M. was a white woman, aged twenty-two; without significant family history; her father and mother were both living and well. She had the usual diseases of childhood with good recovery and no sequelae. Subcutaneous hemorrhages were first noticed when she was about twelve years old, and appeared usually every summer, as large bluish black bruises without pain. During the summer of 1927 the condition was worse than usual, accompanied by considerable bleeding from the gums and profuse menstruation with marked anemia.



When first seen by us on September 13, 1927, her condition was grave, with a marked anemia (see Table) of 40 per cent hemoglobin and 2,430,000 erythrocytes and a blood platelet count of 31,500. She had numerous hemorrhagic petechiae over her body, bleeding from the gums and profuse metrorrhagia. Her bleeding time was thirty-two minutes, coagulation time eight minutes, and her spleen was palpable. She was given two transfusions with considerable improvement in her condition and a splenectomy was done on September 24, 1927. She reacted well from the operation and continued to improve with a decided increase in her hemoglobin, erythrocyte and blood platelet counts. Her leucocyte count was more

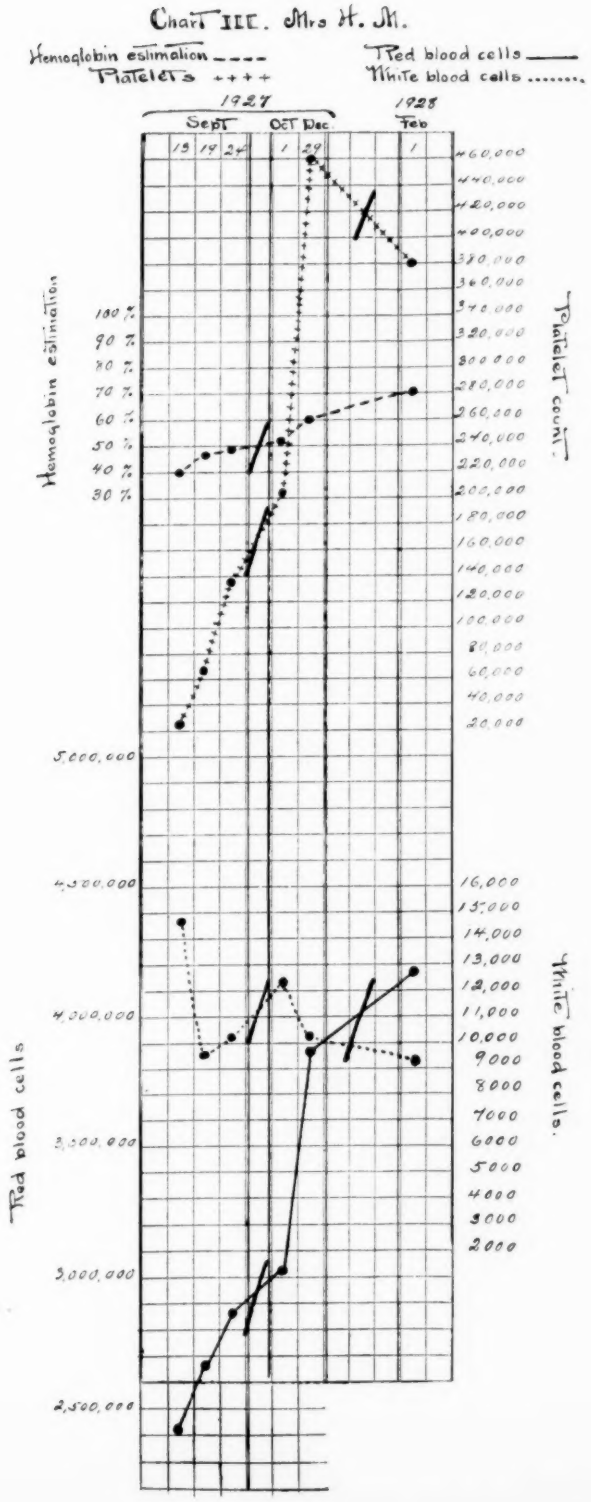


Chart III. Mrs H. M.
Hemoglobin estimation ----
Platelets ++++
Red blood cells ——
White blood cells .....
1927
1928
Sept
Oct
Dec
Feb
19
19
24
1
29
1
Hemoglobin estimation
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
Platelet count
460,000
440,000
420,000
400,000
380,000
360,000
340,000
320,000
300,000
280,000
260,000
240,000
220,000
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
Red blood cells
5,000,000
4,500,000
4,000,000
3,500,000
3,000,000
2,500,000
White blood cells.
16,000
15,000
14,000
13,000
12,000
11,000
10,000
9,000
8,000
7000
6000
5000
4000
3000
2000

or less consistently above the usual normal limits. During the month of January, 1928, she was reported to have had bronchopneumonia, from which she made an uneventful recovery. When last seen on February 1, 1928, her condition was excellent except for a mild anemia.

The spleen removed from this patient measured 15.2 cm. long by 10.3 cm. broad by 5.6 cm. thick, and weighed 322 gm., grossly presenting much of the pathologic picture of the spleen in Banti's disease. Its capsule was smooth and glistening and was not adherent; it was relatively firm and turgid, but collapsed to some extent when sectioned. The pulp was soft and mushy with engorged sinuses and some interstitial proliferation and stringiness of the reticulum. Microscopically there was nothing observed that might be considered characteristic. There was a definite increase in the reticulum and considerable proliferation of the endothelium, with an occasional area of focal necrosis in the Malpighian corpuscles. There was some increase in phagocytosis and in some instances suggestive blood platelet (?) engulfment.

In all three of these cases the outstanding clinical and pathologic manifestations which seemed to be more or less common and characteristic were:

1. The age of the patients; two were young girls about the age of puberty, and the older patient, a woman of twenty-two years, dated the beginning of her purpura back to about the time of her first menstrual period.

2. Subcutaneous hemorrhages of a typical purpuric character, scattered more or less over the entire body, without pain, visceral crises or other complicating diseases.

3. Hemorrhages from the mucous membranes, especially the gums and uterus, particularly at the time of menstruation.

4. Low blood platelet counts, but not excessively decreased.

5. Prolonged bleeding time; deficiency in the retraction of the clot without any significant change in the coagulation time.

6. No significant change in the blood picture, except that of a secondary anemia, with a tendency to a leucocytosis and an increase in the mononuclear and eosinophilic elements.

7. Sufficient enlargement of the spleen to be definitely palpable upon physical examination.

8. The definite improvement by transfusion. Two were apparently cured by splenectomy.

REFERENCE

Greene, C. L.: Purpura. In: Tice, Frederick: Practice of Medicine, Hagerstown, W. F. Prior Co., 1923, No. 6, p. 859.

DISCUSSION

Dr. John W. Gray.—I should like to mention the case of a young child suffering with acute purpura whose life was saved by two transfusions. Within a week following the attack two other children in the family developed scarlet fever. In this case it would seem that this was an individual reaction on the part of the patient. Ordinarily we do not find such reduction in the blood platelets in this type of streptococcus infection.

IMPROVEMENT IN TECHNIC AND RESULTS MADE IN EXAMINING MICROSCOPICALLY BY THE RAZOR SECTION METHOD 2000 MALIGNANT TISSUES*

By Benjamin Taylor Terry, M.D., Rochester, Minnesota

Introduction.—This is a progress report. My previous publication[1] was based on 600 malignant tissues that had been examined perfectly fresh without any fixation whatsoever. The present report is on 2000 malignant tissues. In this 2000 are included many extremely minute specimens on which a year ago I would have failed. The handling of these tiny bits of tissue has necessitated modifications in the technic. The improvements worked out on minute bits of tissue, make it possible to stain the larger specimens more uniformly and successfully. The changes in technic and in the results of the examinations, will be described in this paper.

Advantages.—The advantages pointed out in my previous paper[1] are present in the improved technic. My method is quickly learned, is extremely rapid, uses no carbon dioxid, is inexpensive, noiseless, dependable, and can effect a great saving of time for the surgeon, pathologist, and patient. Moreover, the equipment is simple, light, and so compact that it is readily transported. This makes it possible to employ the method in the operating room as Christeller[2] is now doing. The method will probably continue to be most useful in the rapid microscopic examination of malignant tissues, but it shows also inflammatory and other pathologic changes. It is of great value in selecting blocks to be examined by other methods. It permits the exact orientation of each section and makes the pathologist largely independent of a technician. This last point is of special importance when emergency examinations have to be made. Moreover, microscopic examination by the razor section method does not interfere with the subsequent examination of the same tissue by other methods. Artefacts are few, for tissues can be examined in which the cells are seemingly still alive. If appearances regarded as artefacts in other methods are observed also in razor sections of perfectly fresh tissue, they are probably not artefacts.

Disadvantages.—The razor section method has three disadvantages: (1) If the tissue is not fixed, the sections are not permanent. In this respect razor sections resemble frozen sections of fresh unfixed tissue. To secure permanent specimens it is necessary to fix the tissue and to employ some other stain; e.g., hematoxylin. (2) The cells which are beautifully stained at first, fade quickly. It is necessary, therefore, to examine the sections immediately after staining. the sooner they are seen, the more satisfactory they are. But faded sections can be repeatedly restained. (3) The razor section method while suitable for

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 1928.

the examination of the majority of unfixed tumors is not easily applicable to all. One year ago this limitation in applicability was regarded as the method's greatest disadvantage. With the improvements made in technic in the last twelve months this drawback has already largely been reduced.

Difficult Tissues.—Fresh unfixed tumors and other tissues are difficult to examine for five reasons. They may be hard to cut, stain, mount, examine, or diagnose. Fixation of the tissue overcomes some of these difficulties.

1. Tissues are hard to cut if they contain much calcium or bone, or if they are extremely soft, or small, or friable, or if they are composed of numerous small polypoid masses. But most malignant tissues involving bone have thus far offered little difficulty. Usually they contain masses which can be easily picked out and cut with a razor. Recent improvements make easy and satisfactory the examination of many minute specimens.

2. Tissues are hard to stain if necrotic, or if they contain much unfixed mucus or colloid. But mucus and colloid after fixation give little or no difficulty.

3. A year ago it was said that tissues are hard to mount if very small, or extremely narrow, or ragged, or if the tissue is composed largely of living smooth muscle. The recent improvements make it far easier to examine the narrow strips, and rapid fixation prevents the contraction after staining which formerly was so disturbing when living smooth muscle was sectioned.

4. Unfixed tissues are difficult to examine if red or deeply pigmented. Rapid fixation enables us to cut thinner sections and this usually makes the examination of these tissues much easier. Very porous tissues and sections with rough surfaces are also difficult to examine. The rough sections should be sectioned again to give a smooth surface and the porous tissues are more succesfully stained if the pores are first filled with water to prevent stain entering them.

5. Tissues are at times difficult to diagnose due to inherent difficulties. Borderline tumors often necessitate prolonged study. As sections stained by polychrome methylene blue fade quickly it might not seem that the razor section method would be useful here, although in its favor is the fact that it permits the rapid study of many sections from different parts of the tumor.

Equipment.—There has been no great change in the equipment described a year ago, but to it have been added a camel's hair brush and a needle in a holder. The equipment is still simple and inexpensive. The microscope is the only costly piece of apparatus. There should be on hand a good sharp razor, a fine hone, a piece of compressed cork measuring 10 x 10 x 0.5 cm. or larger, some pins, a good pair of pointed forceps, one or two tumblers, a medicine dropper, a bottle of good neutralized polychrome methylene blue, some clean cover glasses, and microscopic slides, and a strong artificial light with shade. It is convenient to have in addition a few slides of glass or wood in the center of which a round or oval hole about 2 cm. in diameter has been cut.

The Razor.—The kind of razor is less important than its sharpness, for the desirability of keeping the razor as sharp as possible can scarcely be exaggerated. It must be honed whenever it becomes the least bit dull. Stropping is an advantage, but the hone is an essential. Instruction in sharpening the

razor can be easily secured from any good barber. Safety razor blades and microtome knives can also be employed and the Auto Strop Utility Knife has been found useful and inexpensive. Best of all, however, in my experience is the old-fashioned biconcave razor still used by most barbers.

The best cutting board I have yet found is a piece of compressed cork. A table mat made of this material is excellent. Balsa wood also makes a good cutting board. The advantages of this very light wood were brought to my attention by Lieutenant Commander Ragle of the U.S.S. Hospital Ship, Relief.

The Stain.—The stain employed must be good if the results are to be satisfactory. Neutralized polychrome methylene blue made according to the directions given in my paper* a year ago is the best stain I have yet tried. It works with astonishing rapidity on both fresh and fixed tissue. It is also effective on tissues which have been rapidly fixed in formalin, although these frequently do not stain as well as unfixed tissue or tissue which has been thoroughly fixed. Neutralized polychrome methylene blue can be evaporated to dryness without injury and can be preserved as a powder. It goes instantly into solution when distilled water is added and is at once ready to use.

The best light I have tried is a 60 watt white Mazda bulb which has been frosted. This bulb should be supported and shaded. By keeping the lamp four or more inches from the microscope its heat is no longer objectionable.

Slides with holes 2 cm. in diameter cut entirely through them can be easily made out of any soft wood. They are especially convenient for mounting sections which are not of uniform thickness.

It is desirable to keep the razor blade clean and smooth as well as sharp. Wipe the blade off immediately after using. If it is rusted or has clotted blood or bits of dried tissue clinging to it, the surface of the tissue will be scratched and the microscopic picture will not be satisfactory.

TECHNICAL STEPS

1. *Selection.*—When malignant changes are looked for it is important that the right area be selected for examination. If this is not done, the diagnosis will be missed. To select the best area the pathologist should be a good diag-

*Neutralized Polychrome Methylene Blue.

This stain keeps well and acts rapidly, precisely, and differentially on both perfectly fresh unfixed tissue and on formalin fixed tissue. There are five steps in making it. (1) The preparation of stock solutions A, B, and C. (2) The titration of Solution A against Solution C. (3) The alkalinization of B by means of A. (4) The polychroming of the alkalinized B. (5) The neutralization by means of C of alkali already added to B.

The first five steps in detail are as follows:

1. The three stock solutions in neutral distilled water are:

A.	12% K_2CO_3, C.P. anhydrous,	100 C.C.
B.	1% methylene blue, medicinal,	1000 C.C.
C.	10% acetic acid, by volume,	100 C.C.

2. Titration. Determine how much of Solution A exactly neutralizes 1 c.c. of boiling standard Solution C, using phenophthalein as an indicator. Mark this quantity on Bottle A.

3. Alkalinization. Into a 100 c.c. graduate, place that quantity of A, which is equivalent to 1c.c. of C, add enough of B to make 100 c.c., and mix thoroughly.

4. Polychroming. Of the alkalinized methylene blue 25 c.c. are placed in each of four one ounce bottles. These are stood unstoppered in cold water which is brought to a boil in about 10 minutes. Note the time, and remove the bottles one by one 15, 20, 25, and 30 minutes later. Let them cool slowly. The water after reaching the boiling point should be kept boiling while the bottles are in it.

5. Neutralization. To each 25 c.c. of the polychrome stain add 0.25 c.c. of Solution C. Filtration is usually unnecessary and should not be carried out immediately. The four bottles are polychromed differently to permit each worker to make and choose stains which best suit his own taste, light, and work.

Two firms in New York City, the National Aniline and Chemical Company, 40 Rector Street, and Eimer and Amend, Third Avenue at 18th Street, have begun to make the stain here described, and each has agreed to submit samples for my approval before marketing it.

nostician both of the gross and the microscopic changes. Malignant tissues usually depart from the normal. They may be softer, or harder, or more friable. They may be more pink and they are frequently somewhat yellow or contain yellowish specks. By palpation, section, and inspection the areas suspicious of malignancy are selected. From the entire unfixed specimen the pathologist should be permitted to choose for his examination the areas which in his judgment are most likely to yield the evidence sought. The results are apt to be less satisfactory when the surgeon excises the tissue for the pathologist and sends it to him in a bottle of fixative, for after fixation, tissue no longer has its original color or consistence and it is frequently distorted. It becomes often a matter of great difficulty to know how to section such tissue so as to obtain the best results. When tissues are fresh, it is usually easy to

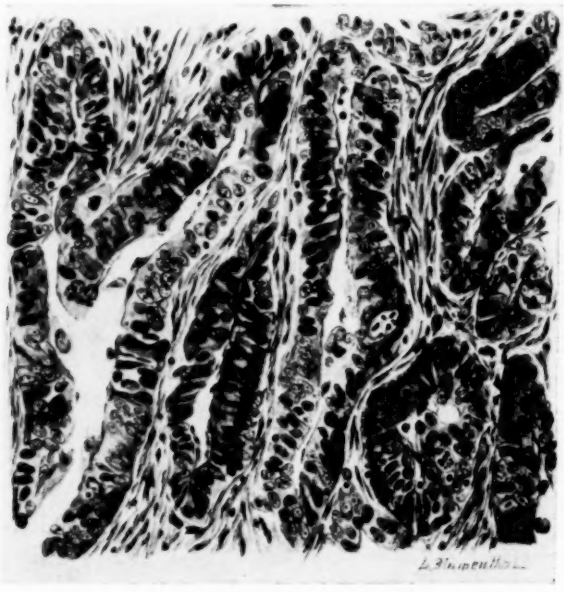


Fig. 1.—Razor section of an adenocarcinoma of the stomach. Stained by N. P. M. B. (Grade 3, Broders)

detect by palpation lymph nodes in which carcinomatous metastases of large size are growing, but after large masses of tissue have been thoroughly fixed in formalin it may take hours to locate a small metastasis that in the fresh state might have been found in a few minutes.

2. *Excision.*—The area selected by the pathologist should, if possible, include the advancing edge of the tumor. Before this area is cut out the next two technical steps must be carefully considered; namely, the immobilization and the slicing of the tissue. The tissue should be so excised as to make both of these steps as easy and as satisfactory as possible. It is important to realize that the block removed for razor section should be thicker than that taken for frozen sections. In the case of frozen sections the tissue is usually thin and flat and the broadest surface is frozen to a table. In sectioning this the microtome knife cuts in a direction parallel to the surface of the table. For razor

sections the broadest and flattest surface is pinned to the cork cutting board, but the razor blade in sectioning is employed perpendicular to the surface of the cork. This means that the tissue to be cut by the razor should be taken differently from that to be sectioned by the freezing microtome. If the surgeon fails to think of this, he usually gives the pathologist sections which are unnecessarily hard to cut and diagnose. A rectangular block measuring 1 x 1 x 0.5 cm. is easily sectioned by the razor but larger or smaller blocks may also be cut. A small block of tissue properly selected is much easier to diagnose than a larger one which is not so carefully chosen.

3. *Rapid Fixation.*—The rapid fixation of tissue in hot formalin is usually not necessary, but at times it is a great aid, especially if the tissues are so soft that sectioning is quite difficult. Large amounts of colloid or mucus in

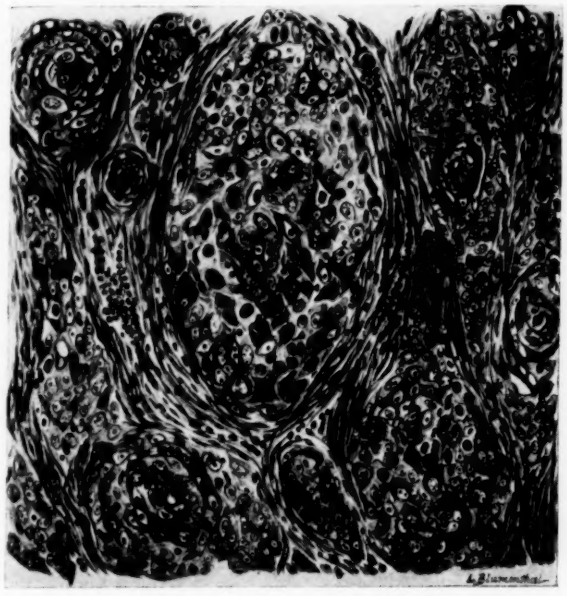


Fig. 2.—Razor section of squamous cell carcinoma of the pharynx. Stained by N. P. M. B. (Grade 3, Broders)

unfixed tissue occasionally make the subsequent staining unsatisfactory. This is due to the fact that mucus and colloid ooze up on the cut surface and so cover the cells that the stain when applied does not reach the cells that should be stained; such tissues are easy to examine after fixation. If difficulty is experienced in sectioning fresh tissue, it is advisable to drop small blocks of this into bottles containing formalin heated from 95 to 98° C. At this temperature small pieces of tissue are quickly coagulated and usually in sixty seconds the consistency is much more suitable for thin sectioning. Fixation is also of advantage where the tissues are deeply pigmented or red.

4. *Immobilization.*—Tissue must not move while it is being cut. The block should have at least one flat surface. The flat surface is pressed gently against the cork cutting board and two or more pins are thrust through the tissue fastening it securely to the board. For one who is right handed it is conven-

ient to pin the tissue 2 or 3 cm. above and to the left of the lower right corner of the cutting board. If the block consists of soft tissue to which is attached firmer tissue or a dense capsule, the tougher or firmer parts if flat should be placed next to the cutting board. If they are not flat they should be so pinned that they are cut after the razor has passed through the softer portions. If this is not done the softer tissue may be crushed.

5. *Wetting.*—Both the tissue and the razor should be wet before the tissue is sliced. Distilled water or clear cool tap water may be employed. By wetting the tissue and razor, friction is reduced and sectioning is made easier and more satisfactory.

6. *Slicing.*—The object is to obtain one or more thin, smooth, plane-parallel slices which shall contain the area that one wishes to examine micro-

Fig. 3.—Razor section of a solid carcinoma of the uterus. Stained by N. P. M. B. (Grade I, Broders)

scopically. The point of the razor rests on the cork cutting board 5 to 6 cm. to the left of the immobilized block. If properly placed, the point is not raised again until the section is completely severed from the block. Before sectioning, the other end of the razor blade is raised and then lowered over the block. By sighting over the back of the razor, one can see how much tissue will be cut off. The tissue may be further supported by sticking forceps in the cutting board in line with the pins and against the right side of the block. By moving the upper part of the forceps forward or backward, they can be made to support the back of the razor blade. This support is a great aid if the hand of the operator is unsteady. In cutting the section the razor is neither raised nor lowered but is drawn along the cork so that the full length of the blade passes through the tissue at each stroke. The first cut is made 1 to 2 mm. in front of and parallel to the supporting pins. If the

razor is very sharp its own weight as it is drawn along will be almost enough to cause it to cut entirely through tissue of average resistance. Unless the tissue is very limited in amount the first piece cut off is discarded. Without changing the position of the razor in the hand, place the point once more in the cork to the left of the immobilized block and sighting over the back of the blade see how thin a piece you can cut by drawing the wet razor again through the tissue. In this way secure a thin, plane-parallel slice which can be stained at once. In slicing be careful not to bring the edge of the razor in contact with the pins.

With practice surprisingly thin sections can be cut with a razor, but thin sectioning can be overdone. If the sections are too thin, they are harder to handle and before staining are best mounted on slides, drawing the slide and the section together out of a glass of water. This usually causes the section to lie flat on the slide. Sections which you are apt to regard as too thick, often give excellent results. If the amount of tissue is small, every section cut should be stained and examined.

Slicing Thin Blocks.—This technic is new and learning it has enabled me to make diagnoses which before were impossible. The cutting board is first moved to the edge of the table so that the hand in cutting will not be hampered in any way. If you are right handed the tissue is placed on the lower right corner of the cutting board and is held against it lightly but securely by a small cork stopper, the small end being down and so resting on the board that the tissue is prevented from slipping toward the operator as he cuts it. Both the tissue and the razor blade are wet. The razor is placed flat on the cutting board and is brought close to the tissue. The back of the razor blade remains in contact with the cutting board but the edge is raised slightly until one can see a small space separating the board from the edge of the blade. The razor is now drawn through the tissue using a single long stroke in which the entire length of the blade is employed. The result is often surprisingly good. At times I have been able to make three or four sections out of one slice that seemed already too thin to section further. Moreover, if the cutting is skillfully done and if little pressure is exerted on the tissue, smooth surfaces are made with each stroke of the razor.

7. *Staining.*—The soundness of the practice of applying stain superficially to one side only of a slice of tissue, has been abundantly confirmed by the large number of sections examined in the last year. But the method has been improved. The former method, which consisted in drawing a section with forceps over a thin film of stain, had several disadvantages.

1. The surface stained is not seen until after it is stained. Irregularities in staining are, therefore, hard to avoid.

2. The method becomes less and less satisfactory the smaller or narrower the section, for it is difficult with the old method to prevent these sections from being stained on both sides.

3. The portion of tissue grasped by the forceps remains unstained. Occasionally the very area that should be studied carefully may in this way escape observation.

4. Some tissues are so friable that thin sections cannot be pulled along without developing cracks into which the stain runs. Sometimes the sections have broken in two.

The problem of how to alter the technic so as to stain successfully sections that are unsatisfactory with the old technic has apparently been solved. Instead of dragging tissue over a thin film of stain, the tissue is now mounted with the side to be stained uppermost. *This side must be smooth.* Beneath the section water is allowed to run so that every crevice is filled. This I refer to as "water sealing," for it prevents stain applied to the upper surface from running beneath and staining the under surface of the section.

Staining Minute Specimens.—The smaller the surface, the smaller the quantity of stain to be used. The surface to be stained should be smooth and moist, but not covered with a thick film of water. If stain is added to a section on which a drop of water is standing, the stain immediately diffuses over the surface of the drop and into the upper layers of the water without reaching the section. Excess of water on the surface should, therefore, be removed by a medicine dropper or by a small piece of smooth filter paper. If the section is extremely small all operations are best carried out under a dissecting microscope and the stain is diluted somewhat before it is applied to the surface by a small pointed camel's hair brush, or by a dissecting needle the point of which has been flattened by rubbing on a hone. The needle is dipped into a thin film of stain and carries over only a very small fraction of a drop when it is applied to the surface of the tissue. The needle has the further advantage that tiny bits of tissue are not picked up by it, as sometimes happens when a camel's hair brush is used. As soon as the stain is applied it is spread quickly over the surface, care being taken not to scratch or injure this surface. The section is then immediately washed with water to prevent the stain from sinking into the depths. By this technic bits of tissue not larger than a pin's head have frequently been beautifully stained.

Staining Frozen Section Remnants.—By using the technic just described I have frequently been able to stain without further section tiny bits of tissue, such as minute carcinomas of the vocal cords, that had already been examined on the freezing microtome. One side of these remnants is smooth and the other rough. All that was necessary for success was to pick out under the dissecting microscope the smooth surface and to stain this.

Staining Larger Specimens.—The technic worked out for minute specimens makes the staining of larger ones easy and frequently more satisfactory than it was before. The more superficial the staining, the more brilliant the result. The section is mounted on a glass slide smooth side uppermost and water is allowed to run under the specimen to fill completely all crevices. The upper surface is drained of its water and the stain is applied with a small camel's hair brush. If the available tissue is limited to one section, the stain may be diluted to avoid danger of overstaining. It is at times advantageous to stain even the larger specimens under a dissecting microscope.

8. *Washing.*—As soon as the upper surface has taken up the stain, it is immediately washed with a large quantity of water. It is convenient to have

a medicine dropper filled with tap water in the left hand while the stain is being applied to the surface of the tissue with the right. Immediately after staining, water is spurted under, then over the stained section. It is a mistake to apply only a small quantity of water to a large section on which an excess of stain has been placed. Under these conditions the stain is merely diluted and may be forced under the section and stain it.

9. *Mounting.*—Nothing new has been learned about mounting the sections. There are two methods. If the section is thin and plane-parallel the stained surface is placed in contact with the cover glass, the cover is turned over, and is then mounted on a glass slide on which a drop of clear water has already been placed. The second method is to be employed when the section is thicker at one end than at the other. The section is mounted on a cover as before, but this cover, instead of being placed on an ordinary glass slide, is turned over and mounted on a slide in the center of which a hole has been cut. The tissue lies in this hole.

10. *Examining.*—It is important to examine the stained specimen at once by transmitted light. If this is not done the staining will appear unsatisfactory, for the sections fade rapidly. The light should be strong enough to transilluminate the tissue, but it is well to avoid using more light than is needed. It is best to cut the diaphragm down until only a small illuminated circle about 3 mm. in diameter is seen where the light strikes the tissue. The intensity of the light may also be regulated by moving the lamp or the mirror, or by lowering or raising the condenser of the microscope. If the section fades before the examination is complete it can be restained one or more times. But the restaining cannot be repeated indefinitely without decolorization, as the stain gradually sinks into the depths and then alters or absorbs so much light that the specimen is no longer properly illuminated. Stained sections can be completely decolorized by placing them in alcohol or in a large volume of formalin. After decolorization sections can be preserved in formalin and restained whenever it is desired.

Diagnosing.—The rapid microscopic diagnosis of razor sections is often easy, but it is sometimes difficult. The harder the diagnosis, the more one should know about the tissue, and the better the technic should be. Before attempting the microscopic diagnosis the pathologist should examine the tissue in the gross. It is far more important to know the exact source of the specimen, its size, shape, color, and consistency, than it is to know the history of the case. Razor sections of fresh unfixed tissue, as well as of fixed tissue, if stained with neutralized polychrome methylene blue, resemble good frozen sections stained with the same stain. Those pathologists who are familiar with the staining reaction of polychrome methylene blue on frozen sections should have no difficulty with razor sections similarly stained. But pathologists who have had no experience with polychrome methylene blue should always control their razor section diagnoses by the method they know best.

Hematoxylin.—The nuclear stain most used by pathologists is hematoxylin. Many of those who have used this dye for years and are thoroughly familiar with it do not feel competent to diagnose sections stained with polychrome

methylene blue. For this reason and also because hematoxylin is not extracted from the sections in the process of clearing to make permanent preparations, it seemed desirable to test this stain. Although my experiments are not numerous, they are encouraging. Razor sections of fixed tissue can often be stained with hematoxylin and be ready for examination in less than sixty seconds. If the section is understained, it can easily be restained. In applying the stain try to keep it from penetrating deeply or staining both sides of the section. To prevent the diffusion of the stain into the depths, razor sections before staining are blotted lightly on smooth filter paper. A thin film of hematoxylin is then obtained by adding a drop or two of the stain to a small piece of filter paper on a glass slide. The section is placed on this film and the stain is allowed to act for that length of time which experience shows is necessary. With the sample of Harris' hematoxylin that I use, thirty to sixty seconds usually suffice, although occasionally better results are obtained with a somewhat longer application. The sections stained with hematoxylin do not show the contrast given by neutralized polychrome methylene blue, but nuclear detail and mitotic figures can be brought out with great sharpness and for purposes of diagnosis these sections are quite satisfactory. They have one very great advantage. They do not fade quickly. They hold their stain well for days or weeks and possibly longer. For those who employ hematoxylin as a routine and who are not familiar with the results obtained by staining sections with neutralized polychrome methylene blue, the sections stained with hematoxylin are often very satisfactory.

In Table I are recorded the results obtained in 1926-28 in examining 600 malignant tissues. The results obtained on fresh unfixed tissue are shown in column 2. In the third column are the results obtained after applying the improved technic described in this paper to the tissues which gave me difficulty when first examined. These early records are not fair to the method, for in the first 200 examinations I was often much disturbed by visitors who came to see the method and who took my attention when it should have been given to recording my findings. If I did not write down at the time of examination that the diagnosis was easily obtained by me, the result was subsequently recorded as a failure. Some of these failures were merely omissions. In my previous paper only the results of examining perfectly fresh tissue were reported. I could not, therefore, include any of my examinations of fixed tissue. But now that my technic is applicable to both fresh and fixed tissue, it has seemed desirable for me to reexamine those tissues which either

TABLE I

MALIGNANT TISSUES EXAMINED	NUMBER OF CASES OF AGREEMENT WHEN MY EXAMINATIONS WERE LIMITED TO FRESH UNFIXED TISSUE	NUMBER OF CASES OF AGREEMENT UPON REEXAMINATION OF THE DIFFICULT CASES AFTER FIXATION
1 - 100	62	99
101 - 200	82	97
201 - 300	91	98
301 - 400	97	98
401 - 500	92	98
501 - 600	96	100

gave me difficulty or on which no record of the success of the examination had been made. This has been done and the results are shown in Table I.

In Table II it is seen that in about 98 per cent of the cases, my razor section diagnoses check satisfactorily with those made by the pathologists of the Mayo Clinic using microtomes. But between the 1701 examination and the 1800, the results check in only 94 per cent of the cases. An explanation may be of interest. The tissues which I receive for examination have at times passed through the hands of a number of men before they reach me. Occasionally as a result of repeated examinations of one small area of malignancy, little or none of it remains for me to find. The six tissues between the 1701 and 1800 examination in which I was unable to confirm the diagnosis of

TABLE II

MALIGNANT TISSUES EXAMINED	NUMBER OF INSTANCES IN WHICH MY DIAGNOSES MADE ON RAZOR SECTIONS CHECKED SATISFACTORILY WITH THOSE MADE BY MAYO CLINIC PATHOLOGISTS ON FROZEN SECTIONS	MALIGNANT TISSUES EXAMINED	NUMBER OF INSTANCES IN WHICH MY DIAGNOSES MADE ON RAZOR SECTIONS CHECKED SATISFACTORILY WITH THOSE MADE BY MAYO CLINIC PATHOLOGISTS ON FROZEN SECTIONS
601 - 700	98	1301 - 1400	98
701 - 800	98	1401 - 1500	100
801 - 900	100	1501 - 1600	98
901 - 1000	99	1601 - 1700	98
1001 - 1100	97	1701 - 1800	94
1101 - 1200	99	1801 - 1900	98
1201 - 1300	97	1901 - 2000	99

malignancy were returned to the hosptial from which they came originally and were carefully reexamined by one of the pathologists, Dr. Wellbrock. In only one of these six cases was Dr. Wellbrock able to find any evidence of malignancy and in this instance the malignant changes were so minute that they were found in one section only, and even then they were so inconspicuous that it was extremely difficult to be sure that they were present at all. My percentage of agreement, therefore, in this 100 although recorded as 94 when compared with the original diagnoses, would be 99 per cent as checked by Dr. Wellbrock when he examined the same material that I examined.

Dependability.—Repeatedly in examining perfectly fresh tissues, diagnoses have been reported to the operating room by Drs. MacCarty, Broders, and Caylor on razor sections before the frozen sections were ready to be examined. Dr. William Carpenter MacCarty when he discussed my paper at the last meeting of this Society said, "I . . . would be perfectly willing to take a bottle of his stain, a couple of pins and a razor and make almost any diagnosis that I would make by any other method. I do not need the microtome. . . . So far as actual recognition of the things which are diagnostic I would as soon have Dr. Terry's preparation as any made with the freezing microtome." Razor sections have been good enough for Dr. MacCarty[3, 4, 5] to make from them his cytologic diagnosis of malignancy and they have enabled Dr. Broders[6, 7] to apply successfully his rules for grading malignancy.

Microdrawings.—The three microdrawings with which this paper is illustrated were made from razor sections stained with neutralized polychrome

methylene blue. For them I am indebted to Dr. Erwin Christeller who not only selected from his own material the areas to be drawn but at every step controlled the accuracy of the reproductions made by his artist. These drawings show, as no words can do, the great nuclear detail that can be seen if the razor section technic is properly carried out. The original drawings in colors are so much like the microscopic specimens that from them alone Dr. A. C. Broders was able without difficulty to grade the malignancy of each of these tumors.

Tested by Others.—The razor section method has now been tested by many physicians. When they have carried out the technic under my supervision and on favorable tissues, no one has failed. Most doctors make good sections at the first or second attempt. Christeller[2] has recently published a very interesting article in which he describes his experiences with the method. He frequently is called upon by surgeons to make immediate microscopic examinations. As most of his diagnoses are secured in thirty to forty seconds, he says the surgeon loses no time as it takes him this long to stop bleeding. In 104 razor section examinations by Christeller[2] the diagnoses thus made checked satisfactorily with those obtained by other methods in 101 instances.

SUMMARY

The razor section method here described has many advantages. It is rapid, easy, inexpensive, and dependable, and it is applicable both to fresh unfixed tissue and to tissue fixed in formalin. No carbon dioxid is employed. Artefacts are few. In studying by the razor section method 2000 malignant tissues a number of improvements in technic have been made. These permit the examination of tissues on which I would have failed a year ago. Thin slices of tissue can be cut still thinner and very minute specimens can now be successfully stained and mounted. The rapid fixation of some tissues in hot formalin has been found advantageous, and razor sections which do not fade quickly have been secured by staining with hematoxylin. By using the improved technic and restudying tissues that have given difficulty the diagnoses made on razor sections of 2000 tissues have agreed satisfactorily with those obtained by the pathologists of the Mayo Clinic using microtomes in 98 per cent of the cases.

CONCLUSION

The new technic has so many advantages that it seems that every pathologist should be willing to give it a fair trial. But no claim of perfection is made. The razor section method should not replace other methods. Instead, it should be used in conjunction with them.

Acknowledgments.—During the last two years I have been privileged to work as a guest in the Department of Surgical Pathology of the Mayo Clinic. Free access has been given me to an abundance of perfectly fresh as well as fixed malignant material. For the splendid spirit of helpful cooperation shown me I am deeply grateful and wish to express here my cordial thanks to all who have assisted me. I am especially indebted to Drs. MacCarty, Broders, Caylor, Wellbrock, Robertson, and Mills.

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VALUE OF LEUCOCYTE COUNTS, ACCORDING TO SCHILLING FORMULA, IN CLINICAL MEDICINE*

By F. W. Niehaus, M.D., Omaha

THE numerical and the differential leucocyte count is recognized as a valuable laboratory procedure; especially in infections it is regarded as an index of severity, and frequently is an important factor in deciding whether or not to perform a major surgical operation. But this measure is not always infallible, and often the leucocyte count does not furnish the anticipated information.

The method to be considered here has been found to minimize greatly these disappointments.

The first attempt to modify the conventional leucocyte count was made by Arneth in 1904, the chief point of which was a classification of the neutrophiles according to the nuclear morphology. His method was rather intricate, and too ponderous and time consuming for use in clinical medicine.

Cooke[1] modified this method, dividing the neutrophiles into five groups. This is a practical method, and furnishes as much information as the more complicated Arneth classification, and is still used by Ponder.

Schilling[2] recently modified Arneth's method. His method is much simpler; in fact, with good preparations, it does not appreciably increase the time and labor of an ordinary differential, and markedly adds to its value. His classification (Fig. 1) of leucocytes is as follows:

1. *Myelocytes.*—The nucleus is round, oval, or kidney-shaped. It is relatively large, vesicular, and stains palely. It is coarsely granular, and usually has a nucleolus. The cytoplasm is pale blue. The granulations are usually delicate, and stain weakly. These are normally found in the bone marrow, but never in the peripheral blood.

2. *Young Forms.*—Normally many are present in the bone marrow, rarely in the peripheral blood. The nucleus is sausage or bean-shaped. It is vesicu-

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lar, and does not stain intensely. There are granulations in the ends of the nucleus. The cytoplasm is like mature cells. At times it is distinctly light blue; the granulations are not so distinct. The cells are usually slightly larger than mature cells. This class is, in part, identical with Pappenheim's "Metamyelocytes."

3. *Staff Forms.*—The nucleus is T, U, or V shaped. The cytoplasm is fully mature. These cells constitute 3 to 5 per cent of the leucocytes of normal blood.

Degenerative staff forms are apparently mature neutrophiles without segmentation. On account of a developmental inhibition, the sausage form does not divide into segments. They are differentiated from the normal staff forms and the young forms by small band-like, often bizarre, twisted and always

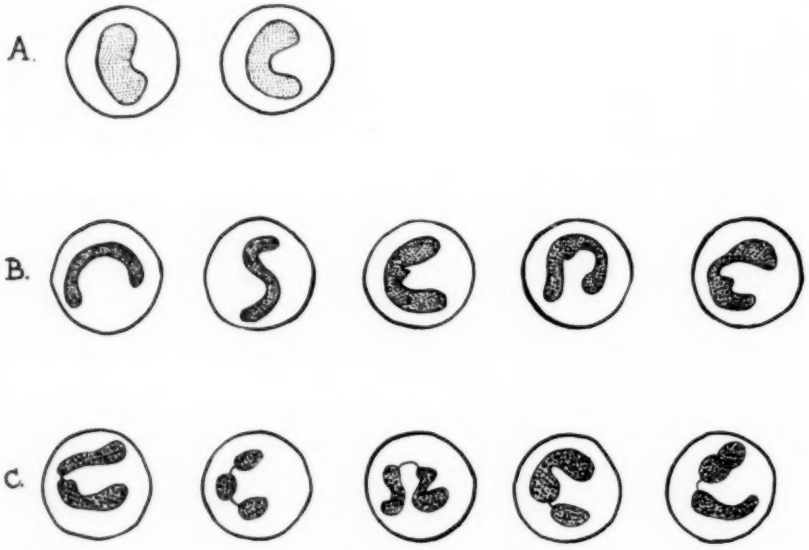

Fig. 1.—Nuclear morphology of neutrophiles; Group A, young forms; Group B, staff forms; Group C, segmented forms.

hyperchromatic (dark, structureless) nuclear form. The granulations either easily overstain or stain feebly and are partially dissolved. These easily break in making smears.

4. *Segmented Forms.*—The nucleus consists of 2 to 5 unequal segments. The latter are united by fine threads. Broader unions (bridges) are temporary attenuations of the nucleus, which are influenced by technic and ameboid movements. Only the former are classified as segmented. About 67 per cent of these are present in normal blood.

The other forms of granulocytes are the *eosinophiles* and the *basophiles*. Cells ordinarily classified as small lymphocytes are the only cells recognized as *lymphocytes*. Those white corpuscles which are usually classed as large lymphocytes, and the transitional cells, are called *monocytes*. The monocytes are

described as at least twice the size of a red blood cell, usually much larger than a granulocyte. The cytoplasm is relatively wide, stains smoky blue to pale violet, and it frequently contains small vacuoles. The nucleus is of a medium size, or relatively large. Its shape is oval, or bean-shaped; it is placed slightly eccentric; the wall is never entirely smooth. It may even be sausage-shaped, or have plump segments.

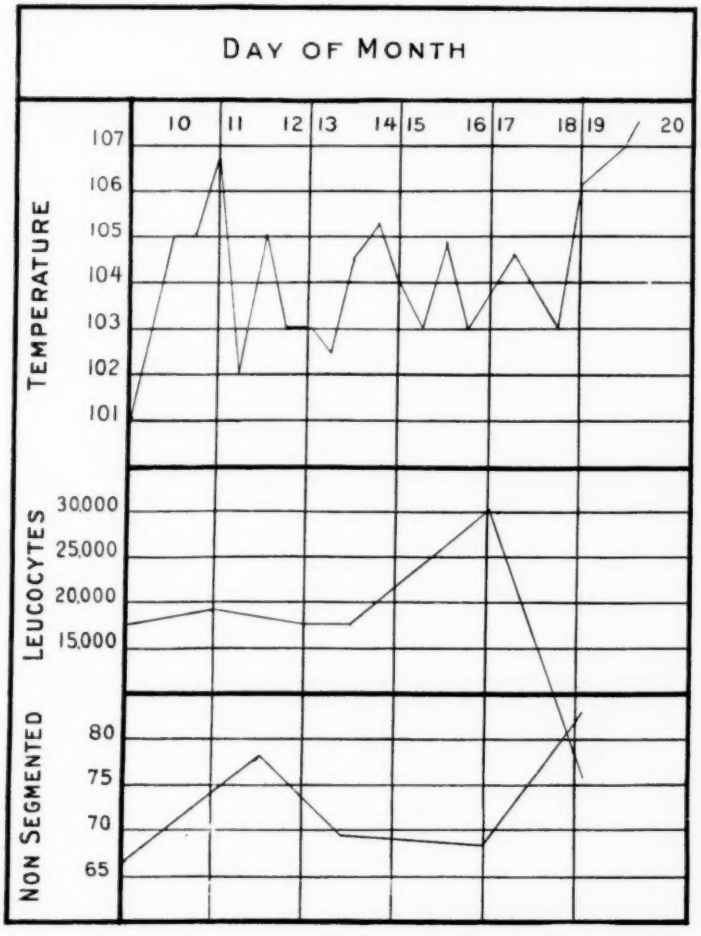

Chart 1.

Schilling further conceives the origin of white corpuscles from three sources (Trialismus), namely: The granulocytes from the bone marrow; the lymphocytes from lymph glands, and lymphoid tissue wherever found; the monocytes from the reticulo-endothelial tissue. The peripheral blood picture reflects the functional status of these organs or tissues, as influenced by their disease, or by their response to exciting agents, as infection. This is further influenced by destruction of leucocytes so that two factors are at work, namely, productive and destructive (Ersatz and Verbrauch).

With pyogenic infection there is a bone marrow stimulation to increased production, and also an increased use of neutrophiles before they reach normal maturity. The greater the stimulus the less mature are the cells thrown into peripheral circulation. So that, first, the number of the staff forms, then the young forms, and even the myelocytes are found in circulating blood.

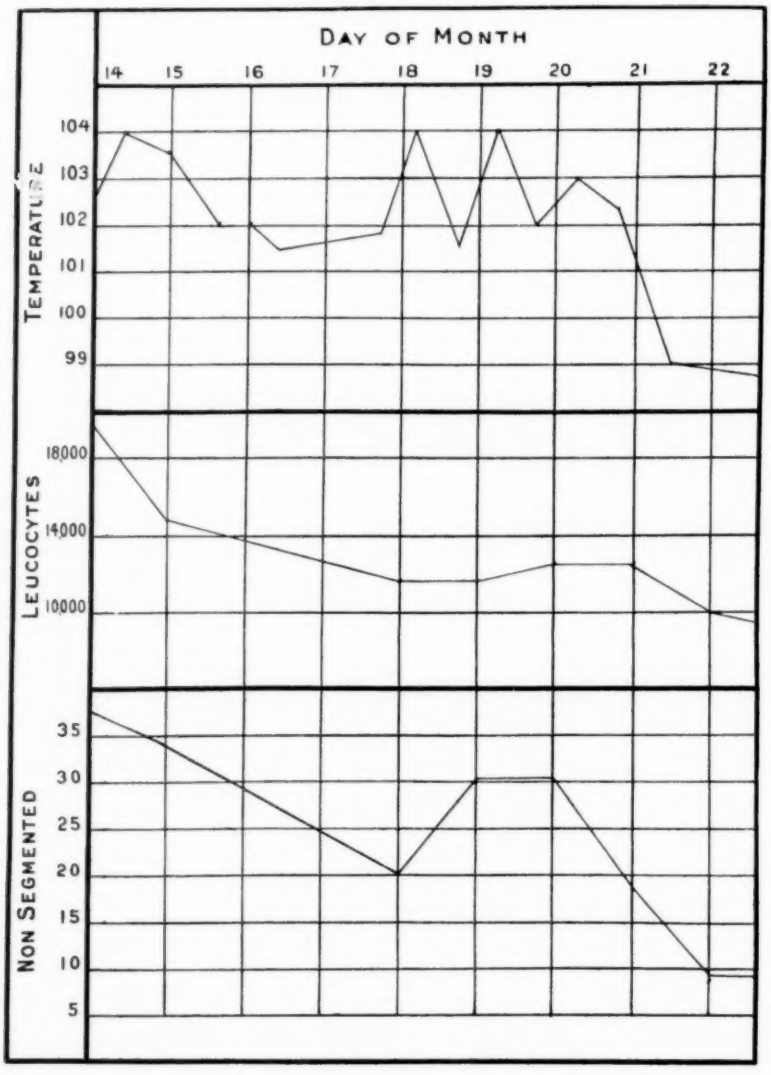

Chart 2.

Coincident with this influx of immature forms, the cells are used (Verbranch) before reaching maturity. In classifying these cells according to the nuclear form and consistency, a marked difference in the nuclei is evident. This is designated nuclear deviation (Kernverscheibung). These are usually written from left to right across a page (myelocytes, young forms, staff forms, seg-

mented forms). As the number of cells belonging to the groups on the left is increased, this condition is referred to as deviation to left (Links verscheibung). Changes enumerated above are regenerative changes. Besides these some diseases and infections exert an influence primarily inhibiting bone marrow. These are degenerative changes.

With this conception in mind, certain blood pictures indicate certain phases of disease or infection.

a. Slight stimulations produce only minor changes with slight increase of staff forms.

b. Moderate stimulus shows a few young forms.

c. Strong excitants cause marked increase of the young and even parent forms (myelocytes).

Ordinarily, the leucocyte count is parallel with the severity of the infection, but with very grave infection a sudden fall may occur. In the latter instance the decrease might be interpreted as an omen of recovery but in reality would be the reverse. The correct interpretation would be evident by proper scrutiny of the individual cells. Undoubtedly many young forms would be present in the blood. This phase concerns only the neutrophiles. When this ends favorably, with a return of nuclear deviation to the right, and decrease of leucocytosis, there is an increase of monocytes. This denotes that the infection has been conquered. The healing phase is evidenced by a lymphocytosis. Eosinophiles also disappear from the blood in severe infection, and their reappearance is the earliest favorable sign. On the other hand, basophiles appear only with severe infection, when the defensive factors are losing ground, and disappear with earliest improvement.

Chart 1 shows the temperature and the blood changes in a fatal case of Staphylococcus septicemia. The increase of the fever, the decrease of the leucocytes, and the increase of the immature forms foretell a fatal termination.

Chart 2 shows temperature and blood changes in a case of pneumonia terminating with recovery. It will be noted that while the number of leucocytes drops early, the percentage of the immature forms does not return to normal until the fever has subsided.

TABLE I

ACUTE APPENDICITIS

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
19,600	0	0	0	0	10	75	15	0		
20,000	0	0	0	0	20	71	9	0		
12,600	0	0	4	0	7	70	19	0		
21,000	0	1	0	0	23	58	18	0		
10,400	0	0	1	2	11	70	16	0		
20,000	0	0	0	0	12	72	15	1		
10,800	0	0	0	0	8	73	19	0		
14,800	0	0	0	0	7	80	12	1		
17,600	0	0	0	2	8	85	5	0	101^{6}	
15,600	0	0	0	1	7	79	13	0		
10,000	0	0	0	4	22	52	18	4	100	Appendix gangrenous

In all cases of acute appendicitis (Table I), there is a significant increase in the number of the younger cells. Its value was particularly emphasized in the cases which have only a slight increase in the total number of leucocytes.

Only those cases of chronic appendicitis (Table II) are included which showed definite chronic inflammatory changes histologically. These are chiefly characterized by a more or less increase of the lymphocytes.

TABLE II

CHRONIC APPENDICITIS

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
8,600	0	0	0	0	10	61	29	0		
13,000	0	0	0	2	4	68	25	3		
7,800	0	0	0	0	4	49	47	0		
10,400	0	0	0	0	5	68	27	0		
10,000	0	0	0	0	3	62	35	0		
10,000	1	1	0	0	4	47	45	2		
6,800	0	2	0	2	3	51	42	0		

TABLE III

ACUTE SALPINGITIS

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
23,400	0	0	0	0	15	69	16	0		
15,000	0	1	0	0	8	58	31	2		
13,800	3	0	0	2	14	57	24	0		
16,000	0	0	0	0	19	55	24	2		
12,000	0	0	0	0	30	50	12	8		
21,300	0	0	0	10	8	77	0	5		

Cases of acute salpingitis (Table III) showed the usual blood picture of pyogenic infection.

TABLE IV

PREGNANCY

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
9,500	0	0	0	0	10	67	21	2		
14,200	0	1	0	1	3	68	27	0		
18,000	0	1	1	1	7	63	27	0		
9,600	0	0	0	0	8	69	22	1		
11,000	0	4	0	0	12	56	28	0		
9,600	0	0	0	1	8	70	19	1		
14,000	0	0	0	1	4	72	23	0		Toxemia
12,000	0	1	0	0	6	66	27	0		
10,000	0	10	0	0	5	72	12	1		
14,800	0	0	1	0	6	83	0	10		

It will be noted that with normal pregnancy (Table IV) the usual leucocytosis is partially due to an increase of the immature forms.

The changes with acute respiratory infections (Table V) were very irregular. Deviation to left was coincident with the onset of a pyogenic process, usually several days after onset of illness.

TABLE V

ACUTE RESPIRATORY INFECTIONS

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
6,000	0	0	0	0	10	32	32	20		Grippe
16,200	0	0	0	0	3	65	28	4		Acute Tonsillitis
11,800	0	4	0	0	2	47	45	2		Bronchitis
11,200	0	1	1	0	17	53	28	0		Pleuritis
9,600	0	0	0	1	3	38	55	3		Throat Infection
17,200	0	0	0	3	5	70	21	1		Acute Bronchitis
12,800	0	1	5	3	12	25	51	0		Grippe
12,200	0	0	0	0	6	62	24	8		Grippe
11,600	0	3	0	0	17	47	25	8		Acute Sinusitis
8,000	0	0	0	0	4	61	34	1		Acute Sinusitis
8,000	1	0	0	0	10	48	40	1		
15,200	0	0	0	0	4	73	23	0		Acute Sinusitis
10,200	0	1	0	1	3	69	26	0		
14,200	0	0	0	0	5	50	45	0		Acute Sinusitis
38,000	0	0	0	0	21	51	28	0		

TABLE VI

ACUTE MASTOIDITIS

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
26,000	0	0	3	2	18	61	1	15		
17,600	0	0	2	4	8	65	1	20		
15,200	0	0	0	1	24	52	8	15		
8,200	0	0	0	0	15	53	28	4		
10,000	0	1	0	0	20	48	27	4		
11,600	0	0	3	1	11	61	20	4		
9,800	0	0	0	0	36	24	26	14		
15,000	0	0	2	0	11	63	24	0		
12,000	1	0	0	2	15	55	16	11		
11,200	0	0	0	2	46	41	10	1		

TABLE VII

FOCAL INFECTION

LEUCOCYTE COUNT	BAS.	EOS.	MYEL.	YOUNG	STAFF	SEGM.	LYMPH.	MONO.	TEMP.	REMARKS
8,800	0	2	0	0	4	64	29	0		Chronic Tonsillitis
7,000	0	1	0	0	8	54	35	2		Chronic Tonsillitis
7,000	0	1	0	0	3	64	30	2		Dental Infection
8,200	0	5	0	0	4	59	32	0		Dental Infection
8,800	0	1	0	0	5	68	26	0		Dental Infection
11,200	0	0	0	0	5	62	23	0		Dental Infection
8,800	0	1	0	0	3	55	41	0		Dental Infection
11,600	3	1	0	2	4	63	8	19		Dental Infection
5,800	1	0	0	0	4	62	28	5		Duodenal Ulcer
11,400	0	0	0	0	4	67	29	0		Duodenal Ulcer
10,000	0	0	0	4	17	32	32	15		Nephritis, Acute, Focal
10,400	0	0	0	4	3	59	32	2		
8,000	0	0	0	2	20	68	10	0		Acute Infectious Nephritis
9,800	0	1	0	2	34	41	18	4		
16,400	1	0	3	3	14	62	15	3		Neuritis
15,600	0	0	1	1	6	68	24	0		
13,900	0	0	0	1	6	70	21	2		Arthritis, Acute, of Hip
9,600	0	1	0	1	0	44	52	2		Arthritis. Val. Heart Disease
6,400	0	2	0	1	2	56	39	0		Arthritis, Chronic
9,600	1	1	0	0	1	51	46	0		Arthritis, Sacroiliac
7,800	0	0	0	1	3	61	34	1		Mucous Colitis
8,000	0	0	0	1	3	59	31	5		Mucous Colitis

In acute mastoiditis (Table VI) there was usually a definite deviation. In several cases with low total leucocyte counts the Schilling differential was particularly valuable.

Chronic focal infection (Table VII) is characterized by an increase of lymphocytes.

SUMMARY

A. Careful technic is absolutely essential:
   1. Smears must be thin and even.
   2. Stains, any good nuclear stain.

B. The method is easily learned:
   My experience is that junior medical students rapidly learned to make reliable counts.

C. Time to make Schilling differential count is practically the same as for ordinary differentials.

D. Results are based on approximately 1000 counts:
   1. Schilling differential is a sensitive, reliable indicator of the severity of the infection.
   2. The grade of infection is often indicated when not apparent by the conventional leucocyte count or clinical symptoms.

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DISCUSSION

Dr. John W. Gray.—How can you differentiate agranulocytic angina from idiopathic aplastic anemias? Is it not possible that the aplastic type of anemia is primary and the throat infection secondary?

Dr. E. R. Mugrage.—We have done some work with this method at the University of Colorado, and we find very similar results to what Dr. Niehaus reports. I would like to ask what technic he uses in counting the cells on the slide. We have been using a method which Dr. Beacom brought out last year by counting across the slide at three different points counting 100 cells at each location.

Dr. F. W. Niehaus (closing).—In regard to the differentiation between agranulocytic angina and aplastic anemia, it seems that the chief point would be the clinical course. In the former there is a normal red blood picture at the onset. The anemia does not occur until later.

Regarding the method of studying the smear, we use the same method as Dr. Mugrage. We pick about four different areas on the slide. Usually one field along the upper, two through the middle, and one along the lower. If I see them bunched up I get a new slide.

THE SPECIFICITY OF BACTERIA TO THE BACTERIOLYTIC ACTION OF CHEMICALS WITH A NOTE ON THIS APPLICATION TO CHEMOTHERAPY*

By Robert A. Keilty, M.D., Washington, D. C.

THE purpose of this paper is to present a report of the apparent specificity or selectivity of antiseptic types of reagents for different bacterial groups with the use of this knowledge for practical chemotherapeusis. Stated the other way around, a bacterial type or a flora freshly isolated will undergo bacteriostasis or bacteriolysis when subjected to different substances, all of which have a definite antibacterial coefficient at different times, irrespective of the antiseptic standard of this coefficient for the particular reagent.

For example, the exposure of a stock culture of staphylococcus to a 1-20 solution of carbolic acid will kill in a given period of time; this is taken as the antiseptic standard coefficient of carbolic acid. Exposure of a number of different cultures of staphylococci, planted directly from wounds or from culture after isolation on blood agar plates, to a much weaker dilution of carbolic acid, say 1-1000, in a nutrient blood agar for twenty-four hours will show a few cultures growing heavily, some retarded, bacteriostasis, and many exhibiting no growth, bacteriolysis. This fact is true for a group of antiseptic substances which I have studied.

Until the World War all work was reported on the standard bacteriolytic coefficient of a given substance as tested out against isolated stock cultures. The memorable work of the Japanese during the Russo-Japanese war, the final development of Dakin's solution after the debate between Sir Almroth Wright and Sir Watson-Cheyne during the World War are still fresh in our minds. At that time I reported the antiseptic value of a number of substances worked out after the method of Watson-Cheyne. Since the War the recognition of the antiseptic value of some of the dyes, the early successes of mercurochrome and the development of a number of new drugs and chemicals have stimulated work along these lines. One of the ideals of medicine is the development of the ever increasing group of specific antibacterial substances.

There are a large group of reagents adaptable to clinical therapeusis which show definite bacteriostatic and bacteriolytic action both in vivo and in vitro. In studying these reactions various dilutions of the drugs from 1-1 to 1-500,000 or even 1-1,000,000 may be made and cultures of various types may be subjected to them for varying times. For example, a given culture may be inhibited by acriviolet, which has a high bacteriolytic coefficient, in a dilution of 1-500,000 in twelve hours. Such a series of tests requires a considerable technical setup with many dilutions and cannot be used practically. When a

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minnesota, June 8, 9, and 11, 1928.

Laboratories of the Diagnostic Center, U. S. Veterans' Bureau, Washington, D. C.

large number of different organisms, maybe of the same type or different types, are used, various dilutions will show varying degrees of bacteriostasis or bacteriolysis in the higher dilutions above the standard bacteriolytic coefficient for that particular substance.

While many methods have been advocated and are in use to bring out these points, about two years ago I began the use of a method which gives some reasonable indication of the antibacterial reaction of a number of substances against a single organism or a given flora. The work was stimulated by the increasing use of mercurochrome, in 1 and 2 per cent solutions, for a wide variety of conditions and with wide variations in results, many of which are most disappointing. The reason for the failures soon became apparent. Too much was being demanded of certain substances, mercurochrome for example, without a specific knowledge of their limitations and without any variation in their makeup, dilution or strength. Thus mercurochrome, a perfectly good drug, bids fair to meet the wastebasket of elimination because we are often asking it to do the impossible.

The method used for this study of selectivity or specificity of a drug against a given infection follows: Plain blood agar plates are used for control isolation of the flora under study. A setup of chemotherapeutic tubes is kept in stock. Plain nutrient agar is prepared in 100 c.c. lots in four-ounce bottles for stock. This is melted, 2 per cent whole blood added as for blood agar plates and the antiseptic reagent added in proportion to the final dilution desired for that particular drug. This is thoroughly mixed and the medium poured and slanted into test tubes 100 mm. by 10 mm. For example, when the final tube is to have 1-10,000 dilution of acriviolet, starting with 100 c.c. of stock agar add 2 c.c. of whole blood, 1.0 c.c. of a standard 1-100 dilution of acriviolet in distilled water. The number of tubes poured will depend upon the amount of work being done. The number of different reagents in different dilutions will depend upon the types and sources of infections with which one is dealing. My work has been mainly on the mouth infections but it may be applied to a number of different sources as will be seen in Table I.

TABLE I

SPECIFIC CHEMOTHERAPEUSIS

TYPES OF CASES STUDIED	
Gingival Infections	356
Nasal Infections	33
Aural Infections	20
Mastoid Infections	2
Eye Infections, Conjunctiva	11
Abscess	11
Rectal Infection, Sinus	1
Pharynx	13
Blood	2
Abdominal Wound Infections	1
Face Infections	2
Bladder Infections	1
Vaginal Infections	3
Prostatic Infections	4
Total Number of Cases Studied	460
Total Number of Cultures, approx.	2500

I have studied a large number of substances including the antiseptics commonly used but I have narrowed the final setup to a group of reagents which meet my needs. After testing a large series of dilutions I have taken 1-10,000 as the standard of dilution for twenty-four-hour incubator reaction with a carbolic acid check of 1-1000 and tricresol check of 1-2000 for my standard of antiseptic coefficient against the organisms under study. If a substance in 1-10,000 dilution shows a constant growth of colonies without even retardation although it may have a constant retardation of growth in 1-100 dilutions it will show an equally disappointing clinical reaction when checked by frequent cultures. On the other hand, the selectivity of substances by retardation of growth against certain flora and the complete inaction in the same dilutions against other flora is a fact which I am unable to explain but which will clinically manifest itself in the same way.

One can have thus prepared on hand a setup of a number of nutrient blood agar slants with different reagents in 1-10,000 dilutions. Material from a case for study is planted directly by loop, streak or swab over the surface of each tube in the setup. Care is taken to have approximately the same amount of material planted in each tube. The tubes are incubated for twenty-four hours at 37.5° C.

In the early work I planted measured amounts of dilutions accurately. While this is advisable in the careful study of antiseptic values for a drug study, approximate amounts will suffice for a practical comparison. The material for study may be anything, pus, mucus, sputum, feces, discharges, skin scrapings, or isolated culture colonies.

Blood agar plates are always planted at the same time for culture control using the same approximate loop quantity. The tubes are read for comparative growth in twenty-four hours and the designations of G., growth; R.G., retarded growth (bacteriostasis); and N.G., no growth (bacteriolysis), used. In a mixed flora one organism may be completely inhibited in its growth while another organism grows profusely. From the standpoint of the case studied this result is charted as growth. The chemical or chemicals in the tube or tubes showing no growth are recommended for clinical use. A number of substances have been tried out and discarded as not practical when they give constant growths in dilutions less than 1-10,000 by this method. Table II shows a group which has been used fairly constantly especially in the gingival work, but which is being changed from time to time to meet conditions. In this table Mer., is mercurochrome; A.F., acriflavine; A.V., acriviolet; G.V., gentian violet; M.B., methylene blue; Agy., argyrol; Met., metaphen; Merph., mercurophen; Phen., phenol; and Tri., tricresol.

It is not my purpose in this paper to report results except in so far as they illustrate the practicability of the method. Table I shows sources of material with the largest groups from the mouth infections. Table II shows the report of 356 gingival cases, seen during the past year, cases showing evidence of gingivitis with mixed cultural floras, in which approximately 2500 cultures have been studied. This table includes the number of cases for each drug used with the number and percentage showing growth, retarded growth and no growth.

TABLE II

SPECIFIC CHEMOTHERAPEUSIS
CHART OF 356 GINGIVAL CASES

REAGENT	MER.	G.V.	M.B.	PHEN.	TRI.	AGY.	MET.	A.V.	A.F.	MERPH.
Total cases	353	354	154	119	34	26	126	355	354	127
Growth	146	168	68	37	8	24	4	8	16	3
Percentage	41	47	44	31	23	92	3	2	5	2
Retarded growth	100	67	37	53	15	2	45	46	66	33
Percentage	28	19	24	44	44	7	35	12	18	25
No growth	107	119	49	29	11	0	77	301	272	91
Percentage	30	33	31	24	32	0	61	84	76	71
Growth and retarded growth	246	235	105	90	23	26	49	54	82	36
Percentage	68	66	68	75	67	100	39	15	23	28

Gingivitis present in one form or another. Mixed bacterial colonies on blood agar plates. Material planted directly from gingival sulci on chemotherapeutic tubes with blood agar control plates. Standard dilution of reagents 1-10,000 with phenol 1-1000 and tricresol 1-2000. Twenty-four hours' incubation at 37.0°C.

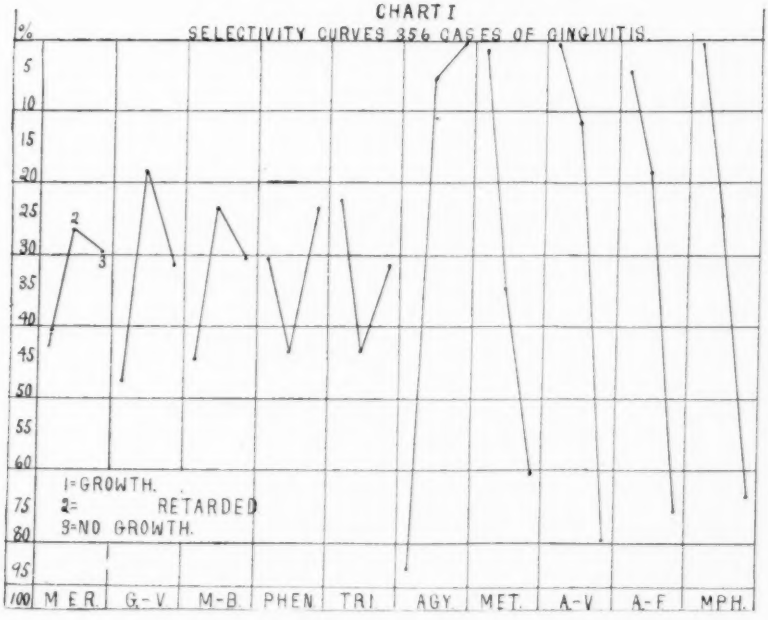
Chart I is a series of curves, showing the comparative standard coefficients of the different reagents against the bacterial flora as it grows from the gingival sulci without reference to the isolated types of bacteria found. The curves show mercurochrome, gentian violet, methylene blue, phenol and tricresol about on a par in the growth, retarded growth and no growth readings, that is a higher percentage of growths than no growth. Argyrol is alone in 92 per cent of growth and never with no growth. Metaphen, acriviolet, acriflavine, and mercurophen are in a group with low percentage of growth and retarded growth and high percentages of no growth.

Chart II shows two curves, A represents the no growths of bacteriolysis, while B represents the growths and retarded growths added together and includes bacteriostasis. The counter position in the curves is made by the grouping of the antiseptic substances.

Chart III is plotted from the six cases taken as illustrations and shows the individual differences in specificity and selectivity of the different reagents. Cases 1, 2, 3, 4, 5 and 6 illustrate the results in individual cases taken at random but with typical variation in readings. Case 1. A-346. Gingivitis. Heavy bacterial flora Streptococcus viridans, nonhemolytic streptococcus, hemolytic streptococcus. Growth on gentian violet; retarded growth on mercurochrome, acriflavine, acriviolet, mercurophen and phenol; no growth on metaphen and tricresol. Case 2. A-493. Gingivitis. Light flora small hemolytic streptococcus with scattered staphylococcus. Growth on gentian violet and phenol; retarded growth on mercurochrome, acriflavine, acriviolet, mercurophen, metaphen, and tricresol. No reagent showed any growth. Does not often occur. Case 3. A-504. Gingivitis. Diffuse culture small hemolytic streptococcus with mucoid pneumococcus. Growth on phenol and tricresol; no growth mercurochrome, acriflavine, acriviolet, gentian violet, mercurophen and metaphen. Case 4. A-622. Gingivitis. Moderate cultural flora with a Streptococcus viridans and a muddy staphylococcus. Growth on mercurochrome; retarded growth, gentian violet, acriviolet, mercurophen, phenol and tricresol; no growth acriflavine and metaphen. Case 5. A-523. Gingivitis. Pure culture heavy Streptococcus viridans. Growth gentian violet and phenol; retarded

growth mercurochrome, acriflavine, metaphen; no growth acriviolet, mercurophen and trieresol. Case 6. A-605. Gingivitis, advanced. Heavy muddy staphylococcus with hemolytic streptococcus, growth gentian violet; retarded growth mercurochrome, acriviolet, mercurophen, trieresol; no growth, acriflavine, metaphen, and phenol. The reason or reasons for this specificity I make no attempt to explain. I do not think the type or types of organisms involved as checked against the individual results is the answer but rather the conditions governing the situation.

There is a practical application of the method described in this paper which is an attempt at specific bacterial chemotherapeusis. When the organisms of a given infection are studied by this method an antiseptic sub-

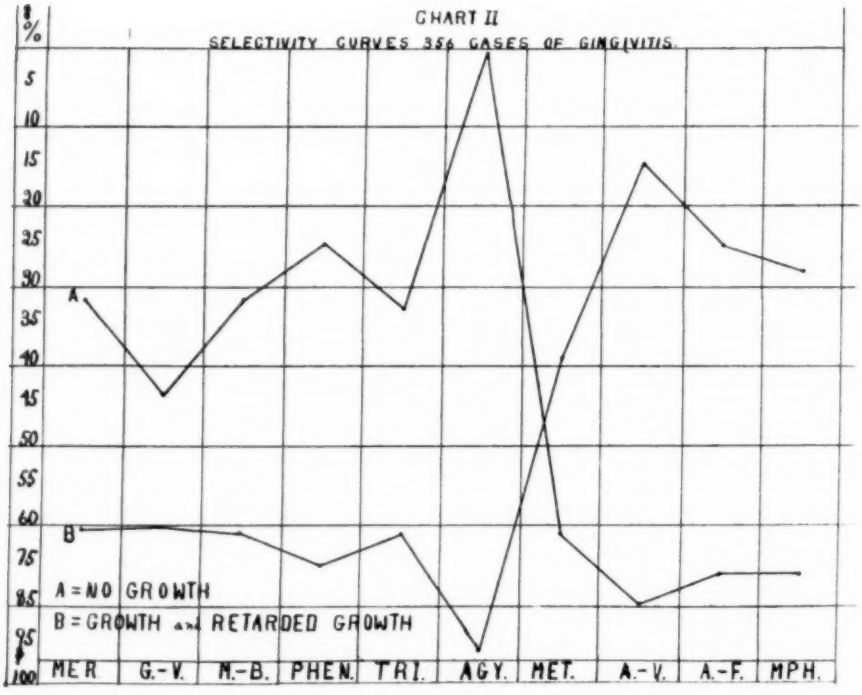


stance, possibly already being used clinically, may be shown to have little or no effect on the retardation of the organisms involved and another substance having an inhibitory reaction may be recommended.

In the treatment of infections by the usual routine methods results are obtained in the course of time. When these methods are improved by selecting an antiseptic group of reagents having a specific bacteriolytic action on the infectious organisms present and when these substances are applied in dilutions just short of irritation and the applications repeated often enough, every hour if necessary over a short period, the length of time of repair is not only shortened but the end-results are most gratifying. The method of application of the drug is just as important as the selection of the drug and the essential feature is that the substance applied must come in constant contact with the bacteria and not be slapped on in a haphazard manner.

The dilution of the drug selected is important. It must be used just short of the point of irritation and this must be worked out by experience. For example, acriviolet in the eye will burn slightly in 1-1000 dilution but it can be used and repeated every two hours for a day or so up to its point of irritation when the interval should be increased. In the nose, acriviolet will be tolerated in 1-500 dilution while in the mouth a 1-100 dilution may be applied to the gums and repeated every two hours.

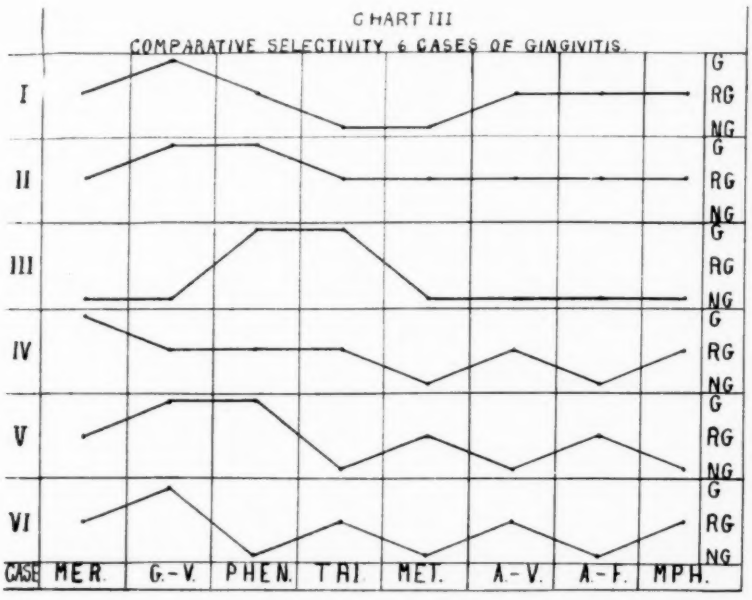
The mercurials, mercurophen and metaphen, which also have a high standard antiseptic coefficient, may be used in 1-100 dilution in the mouth as a local application for a period of days but used as a mouth wash or gargle in this strength are quite irritating and produce a painful soreness which will



promptly clear up when the drug is stopped. On the other hand, 1-1000 mercurophen instilled in 5 c.c. quantities into the urinary bladder may produce such pain that morphine is necessary to control it and yet its antiseptic value is striking. Acriviolet or gentian violet in 1-1000 dilution in the eye may be used while 1-50 may be painted on the skin in erysipelas and repeated every hour without irritating effect.

From these brief remarks and from my experience with various antiseptics over the past two years the following conclusions have been reached: The hit or miss use of any antiseptic, iodine, argyrol, mercurochrome in a straight hundred cases will bring about good results in a high percentage of cases, say seventy-five, will bring indifferent results in about fifteen and will give no results in ten per cent. These results will be surprisingly improved

if the following considerations are worked out for every case: First, the antiseptic substance used must have a reasonably high standard antiseptic coefficient. Second, its selection or specific bacteriolytic action tested out by failure of growth on a nutrient medium with a weak dilution of the drug incorporated in twenty-four hours will pick it out as a more or less specific for this particular infection or infections in the given case. Third, the drug must be used in varying dilutions to suit the part of the body involved and these dilutions must be just short of irritation. Fourth, the drug must be applied at intervals frequent enough to control the infections, as tested by control cultures, every hour if necessary and not once a day or twice a week. Fifth, the drug must be applied by some means which will assure its coming



in contact with the infectious organisms. I think we have too soon forgotten the principles that Carrel used with Dakin's solution.

It must be remembered that all so-called antiseptic substances have a standard bacteriolytic coefficient as tested out on stock cultures. Such tests involve matters of dilution and require a considerable technical setup. Antiseptic substances are used clinically in a more or less hit or miss fashion, and their selection in a given case depends very much upon their popularity with the clinician at the time. Certain antiseptic substances are used on or in every part of the body and for every conceivable condition of infection and necessarily many of the results are disappointing.

CONCLUSIONS

Antiseptic substances may be incorporated in blood agar slants in various dilutions so that direct plants from infected sources may be made and the antibacterial reaction of various substances measured at one time after twenty-four hours' incubation at 37.5° C.

These antiseptic substances seem to have a selection or specific reaction to the growth of microorganisms present in a given infection, vice versa, the same organisms present in a series of the same type of infectious condition will react in their growth differently to the same antiseptic in different cases. For this reason a relatively simple method of procedure is reported in this paper whereby this specificity may be determined and a drug selected for clinical usage which will show in culture definite bacteriolysis to the organism or organisms involved.

When this selection is applied clinically up to its point of irritation, when the application reaches the bacteria and when the intervals between application are short enough to stop reinactivation of the bacteria, the clinical results are most gratifying.

DISCUSSION

Dr. Wm. Thalhimer.—Dr. Keilty's communication is an extremely important one. His method for so easily testing out the susceptibility of organisms to different antiseptics is a definite step in advance.

We have been using the Hygienic Laboratory method for determining the phenol coefficient of disinfectants such as mercurochrome, metaphen, acriflavine, acriviolet, and bichloride of mercury. We found out that the Hygienic Laboratory does not believe that its method should be used in testing out the mercurial antiseptics, but only in testing out disinfectants on a phenol base. In spite of this, for our own interest we thought it would be worth while to find out how some of the commonly used mercurial antiseptics would act under the uniform conditions of the Hygienic Laboratory test. We found that in fifteen minutes a 1-5000 solution of mercurochrome did not kill the typhoid bacilli used. Metaphen killed in dilutions as high as 1-50,000, and bichloride of mercury in dilutions as high as 1-100,000. Under the conditions of this test acriflavine and acriviolet were extremely inefficient. It is only because the results of this study verify in most respects the similar important work of Dr. Keilty that they are being mentioned.

Dr. C. I. Owen.—Have you had any experience with S.T. 37 solution?

Dr. Robert A. Keilty.—This paper deals more with the method than with specific results. In answer to Dr. Thalhimer it is very necessary to procure dyes which are as nearly standard as possible, and in having solutions made for clinical usage they should correspond to the lot number used in the laboratory. The antibacterial action or bacterial inhibition varies under the best of conditions and as nearly as possible the same substances should be used in treatment as in the experiments. Reports on S.T. 37 will appear later.

Dr. F. W. Hartman.—I would like to ask if the bacteria do not develop a tolerance to the various antiseptics so that bacteriolytic action of a particular chemical changes from day to day.

Dr. Robert A. Keilty (closing).—It is necessary to use freshly prepared solutions. The dyes precipitate out rather quickly and lose their antibacterial action proportionately. Bacteria build up a tolerance to chemotherapeutic reagents and if they are to be used over a period of time it is best to use different drugs at intervals.

A SYSTEM OF SPUTUM ANALYSIS FOR THE PRESENCE OF ACID-FAST BACILLI*†‡

By H. C. Sweany, M.D., and Asya Stadnichenko, A.B., Chicago, Ill.

THE finding of acid-fast bacilli in advanced tuberculosis is one of the simplest laboratory procedures, but in childhood, early or latent tuberculosis, it is much more difficult and infinitely more important. In fact, a properly performed series of sputum analyses will give more absolute information than any one type of examination.

Heretofore, animal inoculation has been used in such cases. Recently reliable culture methods[1, 2] have been devised. In both instances, however, there is a considerable loss of valuable time. It is our purpose, therefore, to suggest technical improvements in the direct method to re-establish it, if possible, as the method of choice in laboratory diagnosis.

If such can be accomplished, the advantage both to the physician and the patient is obvious. It will be particularly valuable to Public Health laboratories where large numbers of examinations are made and where the longer procedures are not practicable.

Improvement in the direct method has been made possible by two principles, viz., a development of concentration of sediment, and an apparent increase in bacilli. Other measures are only careful applications of older technical procedures.

Sputum was first concentrated for examination shortly after the discovery of the tubercle bacillus. Biedert[3] as early as 1886 treated a teaspoon of sputum with six to eight drops of sodium hydroxide and examined the sediment. Since then a great many concentration methods have been devised. The various ones may be grouped as the alkali, chlorine, and digestion methods respectively. Only the more important ones will be reviewed here.

Zahn[4] heated sputum with 4 per cent sodium hydroxide and precipitated with normal calcium chloride. Ditthorn and Schultz[5] used 15 per cent potassium hydroxide followed by a liquor ferri oxychlorati. Brauer[6] added ammonia, heated on a water-bath at 50° C. for a few minutes, then added ammonium sulphate and a few drops each of alcohol and chloroform, shook and centrifuged. The tubercle bacilli were found in the middle layer above the chloroform. Hammerl[7] used equal parts of sodium hydroxide and ammonium hydroxide, then decreased the specific gravity with acetone. Mülhaüser[8] used 0.2 per cent sodium hydroxide, heated in a porcelain dish and brought near the neutral point with acetic acid without throwing down the mucus. The sedi-

*This method is an elaboration of the one described by Sweany in the Archives of Pathology (in press).

†Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minn., June 8, 9, and 11, 1928.

‡From the Research Laboratories of the City of Chicago, Municipal Tuberculosis Sanitarium.

ment from this was examined. Bezançon and Philibert[9] used 0.2 per cent NaOH and adjusted the specific gravity of the resultant mixture to facilitate separation of the bacilli.

One of the best alkali methods is that of von Ellermann and Erlandsen[10] who advise incubation of the sputum for twenty-four hours at 37° C. in 0.2 per cent sodium bicarbonate. They poured off the supernatant liquid and heated the sediment with 0.25 per cent NaOH, centrifuged and examined it. Many modifications of this method have been offered, but they possess little advantage over the original. Matson[11] combined the method with the antiformin method and obtained a 12 per cent increase over the latter. Lange and Nitsche[12] used equal parts of sputum and normal calcium hydroxide for three hours in an incubator. The sediment was mixed with 50 c.c. of water and 2 c.c. of ligroine and finely emulsified. It was then heated over a water-bath at 60 to 65° C. long enough to separate sharply the ligroine from the water. The bacilli were found in the layer between the two liquids.

Perhaps the most widely used concentration method is the chlorine or "antiformin" method of Uhlenhuth and Kersten[13] based on the old "Javelle water" method of DeLannoise and Girard cited by Calmette.[14] Its properties depend upon the chlorine liberated from calcium hypochlorite and sodium hydroxide. There should be about 6 per cent chlorine present. From 15 per cent to 30 per cent antiformin is added to sputum and the whole incubated for twenty minutes or more in an incubator. The sediment is examined for tubercle bacilli. There are many modifications of this method.

In addition to the chemical agents that have been used for destroying débris in sputum, Spengler[15] has used an autodigestion method by adding pancreatin to a mildly alkalinized sputum, and incubating.

In conjunction with the use of destructive agents various things have been used to alter the specific gravity of the liquid or the relative specific gravity of the liquid and bacilli. The agents most commonly used have been chloroform, alcohol, and petrolic ether (ligroine). The chloroform causes the bacilli to go to the bottom on top of the chloroform layer; the alcohol decreases the specific gravity when used with chloroform. The ligroine causes the bacilli to rise to the top of the watery layer.

Loeffler[16] uses the antiformin treatment, then adds a few drops of a mixture of one part of chloroform and nine parts of alcohol and centrifuges. Bernhardt,[17] Haserodt,[18] Lange and Nitsche,[12] Kawai,[19] Berry and Smeaton,[20] all recommend the use of ligroine. Many other variations and modifications have been advocated, but for a more complete bibliography, we recommend von Hoesslin's monograph[21] entitled "Das Sputum."

The other principle involved in our method is that of apparent increase of bacilli. Jochmann[22] increased bacilli by mixing 10 c.c. of sputum in 20 c.c. of Heydenglycerine-bouillon. Hesse[23] observed an increase in six to eight hours after seeding sputum on Heyden-agar plates. No increase in direct incubation has ever been made.

The staining of tubercle bacilli has narrowed to a very few methods. The Ziehl-Neelsen method seems to be the standard, but we use the modification of Cooper.[24] Other special stains for granules are those of Much,[25] Kieffer,[26]

Herman,[27] and many others, but as we are not investigating stains and staining methods, we shall not mention them further.

The system adopted at the Municipal Tuberculosis Sanitarium is the result of much experimentation. It will be described in the following pages.

SUPPLIES AND EQUIPMENT

1. Glassware: Round sputum bottles of 1 oz. capacity with wide mouths and measuring 10 centimeters overall, 5,000; plain fifteen cubic centimeters centrifuge tubes, 1,000; glass slides 1 × 3 inches at least, 2,000; 1 gallon earthen jars with handles, 24.

2. Reagents: "Chromegemisch," prepared from commercial sulphuric acid and potassium bichromate, made fresh every week and replenished as they become inactive or empty. Washing soaps and powders. Cooper's carbol-fuchsin stain (3 per cent sodium chloride in Ziehl-Neelsen carbolfuchsin.[28] Acid alcohol (5 per cent nitric acid in 95 per cent alcohol). Methylene blue (Loeffler's diluted to about 30 per cent strength for counter stain). Sodium hydroxide (3 per cent) in a siphon bottle with rubber tube and pinch cock. Lysol or izal disinfectant—sufficient quantity.

3. Apparatus: Four microscopes, equipped with fluorite lenses, good substage lamp and mechanical stages; three staining baths, Corper's[29] or Sweany's[30]; one large International type 24 head shaking machine; one 16 head International type centrifuge; one incubator; one autoclave; twelve galvanized trays 6 inches deep, 8 inches wide; platinum hoops, grease pencils, labels, slide boxes, etc.

Preparation of Glassware.—All glassware is placed in earthen jars, covered thoroughly with fresh and active "chromegemisch" and allowed to stand forty-eight hours when the solution is poured off and the glassware is washed in hot tap water, then in distilled water and dried in a dryer at 220° C. for thirty minutes. The distilled water may seem a useless refinement, but it leaves no salt sediment on drying that follows the tap water washing. The heating does two things: it dries and also destroys all acid-fast form that may be present. When the glassware is used again, it is first placed in trays, covered with lysol or izal, and autoclaved. After this the water tap is turned on over them and all disinfectant removed. They are again washed in soap and water, placed in earthen jars and treated as described above. All faulty or etched pieces are discarded.

Collection of Specimens.—In this part of the work we have the cooperation of the Dispensary Physicians and Nurses. The nurses label the bottle with all necessary data and collect the specimen directly from the patient or leave the bottle at their home with proper instructions. When the specimen is brought in, the cork is pushed in tight, the label is secured by a rubber band, and a triplicate report blank filled out and wrapped around the bottle. The bottle is then placed in a cell in the special carrying case made for that purpose. These specimens are picked up the next morning by a responsible messenger and delivered to the laboratory by one o'clock in good condition. Rarely does a cork come out if they are properly pressed in; even if they do the safety trays will hold them in place.

The System of Examination.—On receiving the specimens at the laboratory, the report blanks are removed from the bottles, checked against the name on the bottle and both bottle and blank are given the same number with a wax pencil. The specimens are then placed on trays, covered with cheesecloth, and incubated eighteen to twenty-four hours in the incubator. This process instead

of decreasing the number of bacilli shows a greater number than by direct treatment. Many times the actual count is nearly double. Much of the mucus and cellular material is destroyed by incubation (see Tables III to V inclusive).

If a bottle is too full of sputum a cork with an opening in it is exchanged for the first cork and the whole tray is covered with cheesecloth and fastened so that the corks will not blow out. After removal from the incubator the bottles are filled with approximately as much again 3 per cent sodium hydroxide making a final dilution to 1½ per cent NaOH. The bottles are then placed in the shaking machine and shaken fifteen to twenty minutes, after which they are incubated thirty minutes and finally poured in the centrifuge tubes bearing the same number as the bottles, and centrifugated at about 3000 r.p.m. for eight minutes. The supernatant liquid is then poured off and the drops of liquid allowed to drain completely. The top of the sediment is then scooped off gently with a platinum hoop and smeared thin on a slide bearing the same number as the centrifuge tube. Should the sediment be too small in amount, an albumin fixative may be used to fix it and make the specimen visible. Should there be too much mucoid material still present, as happens occasionally, the sediment is treated with 5 c.c. of 3 per cent hydrochloric acid, stirred and centrifugated again five minutes. This treatment reduces the sediment again over half.

Through the whole process every specimen, tube, and slide is always kept in order. This is a safety check. The slides are placed in a rack and fixed over the electric bath after which they are stained eight to ten minutes by moderate heating, according to Cooper's method.[24] The slides are then washed in tap water in a special tray by means of a rubber tube attached to a water faucet. Decolonization is carried on to completion (ten to fifteen minutes) and the washing is repeated, the counterstain applied and a final washing given. The slides are then dried over the bath and are then examined until they are found positive or for six minutes or more if they are negative. It has been found by experience that in six minutes about 500 to 1,000 fields will have been covered or about one-thirtieth of the average slide. This is the equivalent of two whole slides by the direct smear method because the sputum is concentrated from 50 to 150 times. It is rare that at least one bacillus is not found in the first six minutes if the slide is positive. If one bacillus is found then a search is made until more are found. It is almost a law to state that we do not find one or two tubercle bacilli alone on a slide. It is possible, but with our method of concentration it is almost always possible to find ten or more bacilli. Once in a long time we find as few as four or five. Less than that we make a special point to re-examine until we find more bacilli or declare it only a suspicious result. *One, two, or three bacilli therefore are only considered as a signal for repeated examinations.* Out of thirty thousand examinations, so far, only once has a report of less than four per slide been made, so that it has been necessary to disregard such finding of acid-fast bacilli as error or accident. Many times we have found from four to ten per slide. After a thorough search and repetition of the examination the specimen usually falls automatically into the positive or negative group and the probability of error is small.

After the slides are found positive, they are registered in a special record book, are labeled and filed for future reference. The reports are then marked, mailed and delivered directly to the proper place, after a duplicate is filed in our records. Within three days from the time the sputum is collected, the reports are received although some may come from thirty miles distant.

To check the various procedures a series of experiments were performed to determine the optimum strength of caustic; to determine whether incuba-

TABLE I

AVERAGE COUNT OF 25 FIELDS OF INCUBATED AND TREATED SPUTUM

NUMBER	TREATED WITH 3 PER CENT HCl	TREATED WITH 3 PER CENT NaOH
1	0.9	4.0
2	10.0	55.2
3	8.7	21.9
4	4.0	20.2
5	9.3	42.6
6	23.7	100.0+
7	24.1	69.8
8	9.5	100.0+
9	1.0 in many fields	1.0
10	15.6	45.1
11	19.3	48.7
12	12.7	38.2
13	37.2	200.0+
14	1.0 in every two fields	2.0
15	4.5	17.3
16	9.0	50.1
17	5.2	29.4
18	16.2	49.5
19	1.9	4.8
20	9.5	37.2

TABLE II

AVERAGE COUNT OF 20 FIELDS OF STAINED SMEARS OF SPUTUM INCUBATED IN TWENTY-FOUR HOURS

NUMBER	CONTROL (UNTREATED)	3 PER CENT HCl FOR 20 MINUTES	3 PER CENT NaOH FOR 20 MINUTES
1	7.2	9.4	21.9
2	5.2	9.0	29.4
3	0.4	11.7	46.0
4	0.5	6.5	25.0
5	2.2	5.3	29.0
6	6.0	12.0	43.9
7	2.4	4.3	19.7
8	2.0	30.1	98.7
9	10.3	—	49.0
10	5.2	—	28.7

tion destroyed any tubercle bacilli; and to test the efficacy of the method in actual practice. Sputums were collected carefully and all work performed in a uniform manner.

Table I shows a comparison of 3 per cent HCl and 3 per cent NaOH as concentrating agents. The NaOH concentrates on the average about five times the HCl.

Table II is practically the same except an untreated control is used for comparison. The HCl concentrates to about half the untreated control. This,

it must be pointed out, is on an incubated specimen that is concentrated from five to ten times by incubation.

Table III shows the effect of incubation on sputum treated with sodium bicarbonate and incubated. There is an actual increase in numbers following the incubation. This speaks well for the von Ellermann and Erlandsen method.

Tables IV and V show that the increase on incubation continues to forty-eight hours and that it does not diminish perceptibly in five days.

TABLE III

AVERAGE COUNT OF 20 FIELDS OF STAINED SMEARS OF SPUTUM TREATED WITH $NaHCO_3$ AND INCUBATED

NUMBER	3 HOURS	20 HOURS
1	5.2	10.3
2	4.0	7.0
3	2.4	3.7

TABLE IV

AVERAGE COUNT PER FIELD OF 20 FIELDS OF TWENTY-FOUR HOUR SPUTUMS INCUBATED TWENTY-FOUR AND FORTY-EIGHT HOURS RESPECTIVELY

NUMBER	24 HOURS	48 HOURS	NUMBER	24 HOURS	48 HOURS
1	11.4	17.9	16	22.9	32.2
2	37.8	44.0	17	25.3	30.0
3	20.5	23.9	18	8.4	10.7
4	18.7	23.0	19	10.4	11.5
5	14.0	27.0	20	6.3	8.7
6	41.4	46.4	21	12.2	19.7
7	14.0	15.1	22	20.5	27.8
8	3.2	7.2	23	17.6	22.4
9	13.7	20.5	24	10.7	17.1
10	5.0	3.8	25	22.5	8.4
11	10.4	8.5	26	10.0	25.1
12	7.3	8.2	27	5.2	7.3
13	5.2	6.0	28	3.1	5.4
14	4.4	2.7	29	11.5	19.8
15	1.0	3.0	30	19.7	38.6

TABLE V

AVERAGE COUNT OF 10 FIELDS OF STAINED SMEARS OF INCUBATED SPUTUM

NUMBER	24 HOURS	48 HOURS	3 DAYS	4 DAYS	5 DAYS
1	9.3	12.5	8.4	10.7	17.1
2	7.2	10.0	25.0	30.0	25.3

Table VI shows the amount of concentration before treatment with 3 per cent HCl on twelve consecutive sputums. By treating specimens 125, 127, and 134 with 3 per cent HCl the concentration was increased to nearly fifty times. It can be stated, therefore, that a concentration of fifty times can be assured. We have found by experience that no more than 7 per cent have to be given the HCl treatment in actual practice.

We have also been able to determine the number of bacilli expectorated per day. A dozen slides were carefully prepared, weighed, smeared in the usual fashion, dried and again weighed. In this manner the actual dry weight was obtained. It was found that the average dry smear weighed 0.3

mg. each. From this weight, the weight of the actual sputum represented can be calculated by allowing a loss of 90 per cent in weight due to moisture, and by multiplying by fifty the average concentration. This calculation shows that about one to two centigrams of sputum is represented on each slide. Taking the number of bacilli per field and multiplying by the approximate number of fields will give the number per slide or per centigram of sputum. This number multiplied by the centigrams per day will give the total number of bacilli per day. The only variables are the number per field and

TABLE VI

CONCENTRATIONS OF SPUTUM

NO.	QUALITY	QUANTITY C.C.	SEDIMENT C.C.	PER CENT	TIMES CONCENTRATED
123	M.P.	4.5	0.08	1.8	56
124	Mucoid	3.5	0.03	0.8	116
125	Mucoid	2.5	0.13	5.2	19
126	Purulent	2.0	0.025	1.2	80
127	Watery	3.0	0.13	4.0	23
128	Purulent	2.5	0.05	2.0	50
129	M.P.	4.0	0.03	0.75	130
130	M.P.	4.5	0.03	0.70	150
131	Mucoid	3.5	0.13	5.0	47
132	M.P.M.	3.5	0.03	0.9	116
133	Mucoid	3.0	0.06	2.0	50
134	M.P.M.	5.5	0.35	6.5	16

TABLE VII

SPUTUM RECORD OF TUBERCULOSIS SUSPECTS AT THE MUNICIPAL TUBERCULOSIS SANITARIUM DISPENSARY

Patient—P. B.		
Result:	1-16-28	negative
	1-25-28	negative
	1-27-28	negative
	1-30-28	negative
	2- 4-28	negative
	2-16-28	negative
	2-24-28	negative
	3-29-28	negative
	3-29-28	+ 1 per field
	4- 2-28	negative
	4- 4-28	+ 1 per field
	4-13-28	+ 1 per field
	4-16-28	negative
	4-19-28	+ 4 per field
	4-25-28	+ 10 per slide
	5- 4-28	+ 10 per slide
	5-17-28	+ 2 per field
Patient—F. B.		
Result:	8- 4-27	negative
	8-15-27	negative
	8-23-27	negative
	8-29-28	+ 5 per slide
	5-17-28	+ 13 per slide
	5-24-28	+ 16 per slide
Patient—K. G.		
Result:	1-30-28	+ 11 per slide
	5- 7-28	more than + 10 per field
Patient—S. B.		
Result:	4- 3-28	+ 15 per slide
	5- 4-28	+ 2 per field
Patient—P. A.		
Result:	9-20-27	+ 5 per slide
	9-27-27	+ 1 per field
Patient—E. S.		
Result:	8- 2-27	negative
	8- 9-27	+ 1 per slide
	8-16-27	negative
	12-22-27	+ 1 per field
	12-30-27	+ 1 per field
	1-11-28	+ 2 per field
Patient—P. M.		
Result:	11-14-27	negative
	3-29-28	+ 11 per slide
	4-12-28	negative
	4-16-28	negative
	5-12-28	negative
	5-25-28	+ 3 per field
Patient—C. H.		
Result:	5- 8-28	+ 3 per slide
	5-22-28	+ 1 per field
	5-28-28	+ 1 per field

the grams per day. For example, if there are two bacilli per field and the patient expectorates 100 gm. per day the total number will be 2000 F. where F. represents the number of fields per slide. This number we have calculated at about 20,000. Therefore, the patient would expectorate 40,000,000 bacilli per day. By using the Roman M for thousand the patient's coefficient will be 40 MM.

Where there are only a few bacilli per slide, the calculation is different. If the examiner searched fifteen minutes and found 12 bacilli, he will have covered 3000 fields or $\frac{3}{20}$ of the slide, as pointed out above. There are therefore 80 bacilli per slide or 800 per gm. If this patient has 10 gm. per day, there will be 8 M bacilli per day. No calculations can be made with less than 10 per slide.

TABLE VIII

AVERAGE COUNT OF 50 FIELDS OF EMULSION OF TUBERCLE BACILLI TREATED FOR VARYING PERIODS OF TIME

NO.	TIME IN HOURS	3% HCl	1% NaOH	2% NaOH	3% NaOH	20% ANTIFORMIN
1	1	6.8	6.9	5.8	5.2	--
	3	6.1	6.0	5.2	4.4	--
	5	5.0	4.3	3.7	3.1	--
2	½	5.9	5.5	4.6	4.6	--
	1	5.1	5.3	4.5	4.2	--
	3	3.9	3.3	3.1	3.1	--
	5	2.8	2.7	2.0	1.7	--
3	1	16.5	15.4	14.9	15.1	12.9
	3	10.3	10.1	8.7	8.1	--
	5	9.5	9.7	7.3	7.0	--
	7	5.7	5.5	4.4	4.2	--
	15	2.7	2.5	2.4	2.0	--
4	1	--	7.3	6.8	6.0	4.8
	2	--	6.0	5.6	5.1	3.9
5	1	--	7.9	6.1	4.8	--
	3	--	5.5	4.9	4.1	--
6	1	--	7.7	6.5	6.0	--
	3	--	7.2	6.0	4.2	--
	5	--	5.2	4.9	3.2	--
7	1	6.5	6.5	--	--	4.1
	2	6.1	5.8	--	--	2.5
	3	5.3	5.0	--	--	--
	5	4.1	4.3	--	--	--
8	½	--	4.2	3.8	--	--
	1	--	3.8	3.5	--	--
	3	--	2.9	3.0	--	--
	5	--	2.4	2.1	--	--
9	½	--	8.3	8.9	7.5	--
	1	--	8.0	7.3	7.0	5.1
	2	--	7.4	6.9	6.3	3.2
	3	--	6.7	5.4	49.1	--
	5	--	5.2	4.7	4.3	--
10	½	--	7.7	7.2	7.1	--
	3	--	5.8	5.1	4.2	--
	5	--	5.0	4.2	3.9	--
	24	--	2.5	1.7	1.4	

In the course of our work we have met with many suspects where only a few bacilli per slide were found or where many negatives were reported for the first few examinations, but they have without exception become frankly positive later.

Table VII reveals the reports on seven such patients. It is a remarkable check on the method. One patient has been under observation for nine years but no tubercle bacilli were ever found by the older methods, yet he was found positive on the first examination made by this method.

In order to check the effect of various concentrating agents on tubercle bacilli, a series of experiments were performed where the bacilli were exposed to varying concentrations suspended in 1-10,000 sodium bicarbonate solution for varying periods of time. The results are charted in Table VIII.

In Table VIII it will be seen that the antiformin treatment is much more detrimental than sodium hydroxide and that 3 per cent sodium hydroxide is more detrimental than 3 per cent hydrochloric acid. *But we feel that 1.5 per cent sodium hydroxide destroys very few tubercle bacilli in the time required to complete the test.* This is obviously no comparison of viability following such treatment.

DISCUSSION AND CONCLUSIONS

We are confident that the system reported concentrates sputum as much as possible without appreciable destruction of tubercle bacilli. The method is simple and can be used by anyone in any laboratory. We have constantly borne in mind time-saving devices. This has been done to make the test as simple as possible and at the same time rapid, because of the large numbers of daily examinations that we have to make.

Objections may be raised against the continued use of glassware because of bacilli that may be carried over. This is not at all valid. By cleaning thoroughly and bathing in "chromegemisch" all organic material is destroyed. Then the heating at 220° C. for thirty minutes destroys all acid-fast forms. This last step alone is nearly sufficient. We have heated six tubes in which large numbers of bacilli were present and on examination, after heating, found no acid-fast bacilli.

We feel that Cooper's staining method has gained a permanent place in laboratory diagnosis. We have not found that it gives any false positives if used as the author prescribes and it surely renders the bacilli more distinct.

The question of actually increasing the bacilli in the digestion of sputum is one that cannot be answered positively. It seems from actual count that they increase from two to many fold. This has been done on carefully divided sputum and after a count of many fields. From the appearance of the bacilli it seems that actual division is taking place because they are so frequently found in "nodal" or vegetative forms and in pairs. We must also not overlook the possibility of a sprouting of the granular forms. This does happen in certain specimens according to our own observation. How general it is remains to be proved. It may help to account for the increase in bacilli. Furthermore, the diagnosis of old suspects seems to support this theory.

This method is obviously not intended for sputum with large numbers of bacilli although it is almost as simple. As a method for the examination of suspects, however, it has no equal at the present time.

SUMMARY

1. A practical concentration method has been described that concentrates sputum more than fifty times.

2. The important feature is autodigestion of the sputum with apparent increase of bacilli.

3. It approaches the accuracy of animal inoculation in the early diagnosis of acid-fast bacilli in the sputum.

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DISCUSSION

Dr. E. D. Downing.—We still have to depend upon the tubercle bacilli for the absolute diagnosis of tuberculosis in the chest. With all the improvements in the x-ray and in clinical methods, the work reverts to the tubercle bacilli. We must be very careful about finding the tubercle bacilli. We are getting sputum reports as positive in cases of bronchiectasis. There seems to be some increasing source of error somewhere. There seems to be some source of error in the cases they are sending to us about other organisms in the sputum that will remain red. We teach our technicians that everything red is a tubercle bacillus; we run into diphtheroid organisms in the sputum that are acid fast, and we get a report of tuberculosis. It is the same with textbooks giving four or five different methods of staining. It seems that these methods have sources of error in them. This complaint is not so much for doctors as it is for technicians.

For some time we have made routine cultures on Dorset's egg medium. It is surprising that, possibly one in ten, you will get a growth of tubercle bacilli in three weeks whereas you let the guinea pig run six weeks. I doubt if there are many laboratories that make a routine culture. In sanitarium work all the material that comes in to us we run through on Dorset's egg medium. I was glad to hear Dr. Sweany speak of the live germs in the sputum. The latest impression is that these germs are dead and that they are not dangerous to other people. I think that is one of the most important things that the tuberculosis worker has to impress on his patient; that these germs they spit up are alive and not dead.

Dr. E. R. Mugrage.—We have a large number of indigent patients in the University of Colorado Clinic. In the past we have also had the large numbers attending the city clinic. While we were taking care of that sputum work, we found that the antiformin concentration method was not satisfactory. In fact the late Dr. Todd insisted that a proper selection of the particle of sputum to be examined was of more value than the antiformin concentration method, and he insisted that we spend the time that we would devote to the concentration method in the proper selection of a caseous mass. At the present time we utilize a paper dish which we coat with paraffin colored with lamp black. With that background we are able to pick out suitable particles with the saving of a great deal of time.

Dr. Robert A. Keilty.—It seems to me that these two papers are very timely from the economical point of view. In laboratories with many routine requests for animal inoculations the substitution of cultural methods, which will give as many positive results as animal inoculations, will mean a financial saving. Just last week I saw a bill for $127.00 for sixty-seven guinea pigs.

Dr. Henry C. Sweany (closing).—In regard to the technicians that do this work. We have two that have been doing it for over six years. I will put them up beside anyone else for speed and accuracy.

I did not mean that we do nothing else but concentration tests. Our routine work is carried on by the routine smears as usual. We feel this concentration of the sputum that we have attempted is bringing out more positives than ever before.

A PHOTO-ELECTRIC HEMOGLOBINOMETER*

Clinical Applications of the Principles of Photo-electric Photometry to the Measurement of Hemoglobin†

By Charles Sheard, Ph.D.,‡ and Arthur H. Sanford, M.D., Rochester, Minn.

MANY methods have been devised and used, both in research and clinical practice, for measuring the amount of hemoglobin in the blood. Many critical reviews and comparisons of these various methods have been made, and the advantages and disadvantages, points of accuracy and inaccuracy, and the like, have been pointed out and discussed. It would appear to be of slight if any value, therefore, to rehearse much of what is contained in the literature on this subject.

The method of choice, so far as accuracy is concerned, is the determination of the oxygen capacity of the blood after the manner described by van Slyke. One serious objection to this method is that each determination consumes considerable time, especially if check readings are made. At best, therefore, one person can make, in a day, few determinations of oxygen capacity and hence of the amount of hemoglobin in grams for each 100 c.c. of blood. The apparatus and method are not likely to be used in a laboratory or institution desirous of making as a routine a large number of determinations on the content of hemoglobin. Neither, on the other hand, is the small laboratory likely to be warranted in maintaining the necessary equipment and technical force.

Spectrophotometric methods are very accurate when employed by those thoroughly familiar with and experienced in such optical procedures. The investigations of Williamson, who used spectrophotometric methods in his work on the effects of age and sex on the content of hemoglobin, have been quoted frequently in the literature and his data on the grams of hemoglobin for each 100 c.c. of blood have served as an excellent standard and basis of comparison. Davis and Sheard have reported, within the last two years, their investigation on the spectrophotometric determination of hemoglobin, making their readings at wave-length 542 millimierons using blood diluted 1:200 with a 0.1 per cent solution of sodium carbonate. They report an average variation of 1.3 per cent from the determinations made by the oxygen capacity method of van Slyke. Kennedy also has described recently a method of using selective light filters in connection with a Duboscq colorimeter in measurements on

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‡The author has applied for patent rights covering the use of photo-electric cells in the measurement of hemoglobin and the general principle of the application of such methods with selective spectral filters to the determination of the amounts of unknown substances in solutions. This application for patent rights has been made in order to control the development, accuracy and general serviceability of such devices so that those who acquire apparatus involving the principles disclosed may secure satisfactory equipment. Assignment of any rights granted will be made to The American Society of Clinical Pathologists.

hemoglobin. Spectrophotometric methods are very accurate in general and the technical procedures are fairly simple but considerable experience in such procedures is highly essential. All spectrophotometric methods suffer in accuracy from retinal fatigue, condition of adaptation of vision and, in particular, from retinal sensitiveness and the inability to determine absolute equality of the two portions of the field under observation. Some of these points of inaccuracy can be overcome only to introduce other sources of error which may be as great or even greater.

The simpler and commonly used clinical procedures and apparatus have been criticized for one reason or another, chiefly, however, because of the variability of standards and the fact that such standards usually do not accurately reproduce color in its three attributes, namely, relative luminosity (brilliance), dominant wave-length (hue), and purity (saturation). The Dare method, for example, while it is simple, is not accurate if the hemoglobin content is above 65 per cent and determinations in grams of hemoglobin for each 100 c.c. of blood cannot be made accurately. It is now almost universally agreed that hemoglobin should be reported in grams for each 100 c.c. of blood rather than in terms of ambiguous percentages which are based on some arbitrary standard taken as 100 per cent.

All methods in which the hemoglobin is converted into acid hematin either have very changeable standards or, if such standards of glass are used, the color is not an exact match for the acid hematin. Osgood and Haskins have devised an excellent acid hematin method, employing a colorimeter for purposes of comparison. Their artificial standard, however, while permanent, varies with temperature so that, at the time of each determination, the temperature of the standard must be taken and a table of corrections consulted in making the final calculations on the content of hemoglobin. Newcomer's method involves the use of a yellow glass filter and, while it is one of the most commonly used methods, it suffers from the inherent difficulty of color matching.

The ideal method obviously would be one which involves all the advantages and points of accuracy and eliminates the disadvantages and sources of error of other methods. While such an ideal method, in every particular or detail, has not been reached by us in our investigations thus far, we believe that we have devised and are now perfecting an ensemble of apparatus and a method of procedure which approximates in accuracy the oxygen capacity method of van Slyke and which eliminates the inherent difficulties of spectrophotometry, color matchings, variability of standards and the like by the use of a sensitive mechanical eye, the photo-electric cell. This cell does not suffer from the sources of error and inaccuracy of the human eye or of different observing eyes. We believe, therefore, that the application of photo-electric photometry to the determination of the content of hemoglobin affords a method which, while it may appear complicated in its theoretical phases, can be standardized readily and accurately anywhere and at any time, is very rapid, and is accurate (so far as results thus far obtained indicate) to within about 4 per cent (as its maximal error) when compared with the findings by the

oxygen capacity method of van Slyke; it can be operated successfully by any technician, and it eliminates errors due to the so-called personal equation and the errors inherently present in colorimetry and visual spectrophotometry.

THE PHOTO-ELECTRIC HEMOGLOBINOMETER

Fig. 1 is a reproduction of a photograph of the photo-electric hemoglobinometer proper, the galvanometer, batteries and accessories being omitted. A semidiagrammatic or cross-sectional sketch of the instrument is shown in Fig. 2. The ensemble consists essentially of two compartments (*A* and *B*), connected by a brass tube. Compartment *A* contains the source of illumination and accessories; compartment *B* carries the photo-electric cell, selective spectral filter and absorption cell for the solution of blood to be tested.

Source of Illumination.—The source of light is an automobile headlight lamp operating on six volts. The lamp is fitted into a device which permits of lateral and vertical adjustments so that the filament of the lamp can be centered with reference to the iris diaphragm and shutter and the collimating

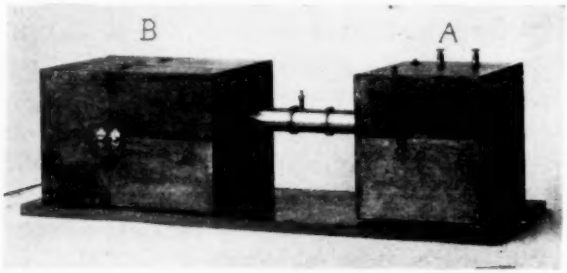

Fig. 1.—Reproduction of photograph of the photo-electric hemoglobinometer. Compartment *A* contains the source of illumination. Compartment *B* contains the photo-electric cell, selective filter and cell for holding the diluted sample of blood.

system of lenses and water cell contained in the brass tube connecting the compartments *A* and *B*. The end of the box *A* can be opened in order to adjust the position of the light source or to vary the size of the iris diaphragm. A shutter release, led out through the side of the compartment *A*, enables the operator to regulate the periods of illumination of the photo-electric cell in compartment *B*.

Water Cell.—The water cell in the tube connecting the two compartments is inserted for the purpose of removing in considerable amount the infra-red radiation (popularly called heat rays) from the source of illumination after passing through the lens in compartment *A*.

Photo-Electric Cell, Light Filter and Absorption Cell.—Compartment *B* is divided into two portions. The part at the left end of Fig. 1 contains the photo-electric cell and is made light proof to all radiation other than that received from the source of illumination after passing through the lenses (compartment *A*) and water cell (connecting brass tube) in addition to the contents of the absorption cell and the selective spectral filter (compartment *B*). The door to the right hand portion of compartment *B* can be opened to allow

the absorption cell and contents to be placed in front of the selective filter or in the optical path of the source of illumination.

The photo-electric cell, which proved most satisfactory and very constant, was obtained from the Research Laboratories of the General Electric Company. This cell has a light-sensitive coating of cesium. Such photo-sensitive substances, when exposed to light, emit electrons. If the photo-sensitive film is connected to the negative terminal of the high voltage or B battery (150 to 200 volts) and the inner electrode is connected to the positive terminal and the photo-electric cell is then exposed to light, the electrons

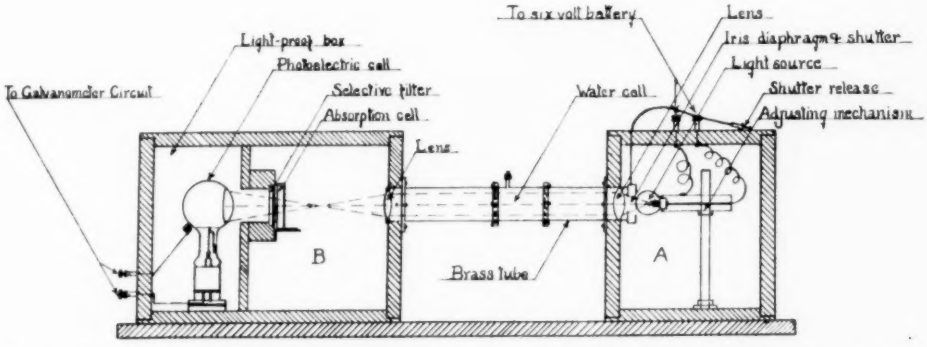

Fig. 2.—Diagrammatic sketch (cross-section) of the photo-electric hemoglobinometer, showing the essential features of the ensemble.

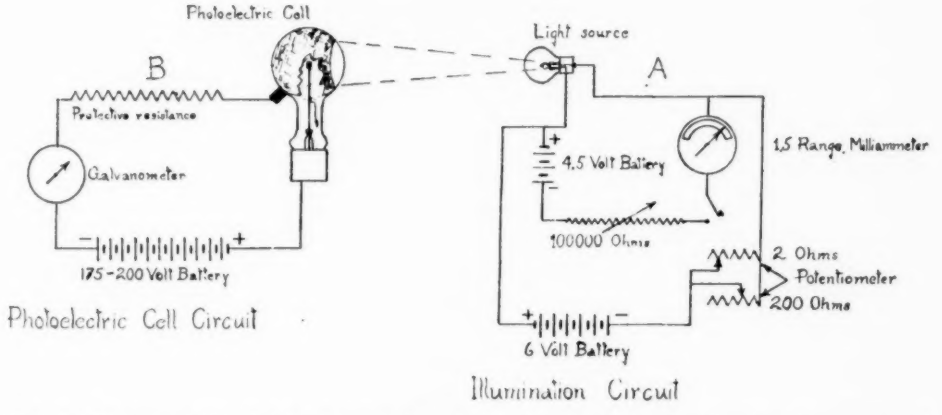

Fig. 3.—Diagrammatic sketch showing the electrical connections for keeping the source of illumination constant (*A*), and the electrical circuit containing the photo-electric cell (*B*).

emitted under the action of the incident light will flow through the cell, thereby setting up a current which will be indicated by the deflection of the galvanometer.

The selective spectral filter which we have used is known as Eastman No. 74, and is the same filter as was used by Sheard and Davis and also by Kennedy in colorimetric methods for the determination of hemoglobin.

The absorption cell is of the type commonly used for spectroscopic and spectrophotometric determinations. Its dimensions are 50 mm. high, 30 mm.

wide and 10 mm. internal depth or thickness. The only dimension of significance is that of depth, which should be as accurately uniform as possible in a group of absorption cells which would be used in a routine examination of a large number of samples of blood.

Electrical Circuits.—Fig. 3 contains additional diagrammatic sketches of the electrical connections and adjustments for both the illumination circuit and the photo-electric cell circuit. The source of illumination was operated on a six-volt storage cell system, two such storage cells being connected in parallel. It is very essential that the voltage across the lamp which serves as the source of illumination be maintained constant throughout the routine of reading the deflections of the galvanometer included in the photo-electric cell circuit.

Control of Current in the Source of Illumination.—The potentiometric scheme shown in Fig. 3 (*A*) illustrates how this may be accomplished. A 4.5 volt battery (dry cells) is joined in series with a 1.5 range milliammeter and a resistance of 100,000 ohms. This 4.5 volt battery is so connected as to be opposed to the electromotive force of the main 6 volt battery. By means of the potentiometric device, consisting of 200 ohms (coarse adjustment) and 2 ohms (fine adjustment) in parallel, it is possible to make adjustments such that no flow of current will be indicated on the milliammeter. As long as a null reading of the milliammeter exists (which is determined by an occasional closing of the switch in the circuit containing the ammeter, the 100,000 ohm resistance now being eliminated), the operator is assured of the fact that the voltage across the source of illumination (and therefore the current flowing through the lamp, if the resistance of the lamp filament remains constant) is constant.

Control of Constancy of Illumination.—This device for the determination of the constancy of voltage across the terminals of the source of illumination, however, does not obviate or compensate for any variations which may arise in the circuit containing the photo-electric cell and the high voltage battery. Variations in this circuit may occur because of electrical leakage, which may vary with conditions of the weather, slight changes in sensitivity of the galvanometer and the like. This difficulty can be overcome readily by taking some arbitrary value of deflection of the galvanometer in the photo-electric cell circuit (such as 400 mm. for example, as in our work) when the absorption cell is removed from its position in front of the selective spectral filter in compartment *B*. If the reading of the galvanometer is either greater or less than the fiducial number, the iris diaphragm (compartment *A*) is either opened or partially closed and so adjusted as to give a reading on the scale of the galvanometer approximately equal (within a few millimeters) of the arbitrarily chosen standard.

Galvanometer.—The galvanometer in the photo-electric cell circuit is a Leeds and Northrup high sensitivity galvanometer, No. 2285 c, having a resistance of about 550 ohms, a period of about fourteen seconds, an external critical damping of 20,000 ohms and a sensitivity of about 1×10^{-10} ampere for each millimeter of deflection. The protective resistance (Fig. 3, *B*) amounts to several thousand ohms (10,000 to 20,000 ohms).

PREPARATION OF THE SAMPLE OF BLOOD

We have tried various dilutions of blood and various types and thicknesses (internal depth) of cell. Experience indicates that the following simple technic is entirely satisfactory. The blood must be laked and the resultant solution of blood (in which the hemoglobin is wholly oxyhemoglobin) must be very clear. Therefore, the proteins must be kept in solution. A 0.1 per cent solution of sodium carbonate is a satisfactory diluent. To cover the range of grams of hemoglobin (extending from conditions of polycythemia to severe anemia) it was found, after many trials, that a dilution of 1:200 was satisfactory in that such a dilution of blood afforded a sufficient range of readings on the photo-electric hemoglobinometer when absorption cells of 0.1 c.c. thickness were used. Such a dilution can be made by adding 0.1 c.c. of whole blood to 20 c.c. of 0.1 per cent solution of sodium carbonate. The diluent is carefully measured with a burette and placed in a suitable container. We make use of 50 c.c. lipped centrifuge tubes for this purpose: such containers make the filling of the absorption cell an easy matter. The pipette in which the blood is drawn obviously must be accurate: we use a serologic pipette, graduated in thousandths of a cubic centimeter, drawing the blood (taken from the vein) exactly to the 0.1 c.c. mark. All blood is carefully wiped from the outside of the tip of the pipette and the contents blown at once into the solution of sodium carbonate. The pipette is washed out four or five times by drawing up and releasing the diluting fluid. The blood is laked instantly by shaking the tube for a few seconds. The solution of blood is then ready for insertion in the cleansed absorption cell and for obtaining the reading on the scale of the galvanometer in the circuit of the photo-electric hemoglobinometer.

GENERAL PROCEDURE

By way of outlining the routine of procedure which we have adopted we may say that the blood is drawn and diluted in the manner described in the preceding paragraph. The sample of diluted blood is placed in the absorption cell. This cell and contents are then ready for insertion in a suitable containing block so that they rest directly in front of the selective spectral filter. When the necessary adjustments of the illumination circuit have been made and the fiducial reading of the galvanometer secured (with no absorption cell present), the sample of diluted blood is inserted in the instrument and the galvanometer deflection, which measures the amount of transmitted light, is read and recorded. From the chart showing the relationship between the grams of hemoglobin, as determined by the van Slyke apparatus and method, and the deflections of the galvanometer, the grams of hemoglobin for each 100 c.c. of blood are read off and recorded.

THEORY OF THE PHOTO-ELECTRIC PHOTOMETER AND SPECTROPHOTOMETRY AS APPLIED TO THE QUANTITATIVE DETERMINATION OF HEMOGLOBIN

In the presentation of the theoretic and practical principles underlying the applications of the photo-electric photometer to the quantitative determination of hemoglobin, it is fundamental that the laws of spectrophotometry

be presented and discussed, since both methods afford a means of measuring the transmitted (or unabsorbed) light that passes through any solution under test. A further reason for the presentation of the facts underlying spectrophotometry is the use we have made of this method by way of checks on the determinations made with the van Slyke apparatus and the employment of these spectrophotometric determinations in evaluating the readings of the photo-electric hemoglobinometer.

SPECTROPHOTOMETRIC DETERMINATIONS OF THE ABSORPTION OF DILUTED BLOOD AND OF THE TRANSMISSION OF THE SPECTRAL FILTER

In Fig. 4, Curve 1, is shown the two oxyhemoglobin bands of blood; the alpha band with its center at 578 millimicrons and the beta band with its

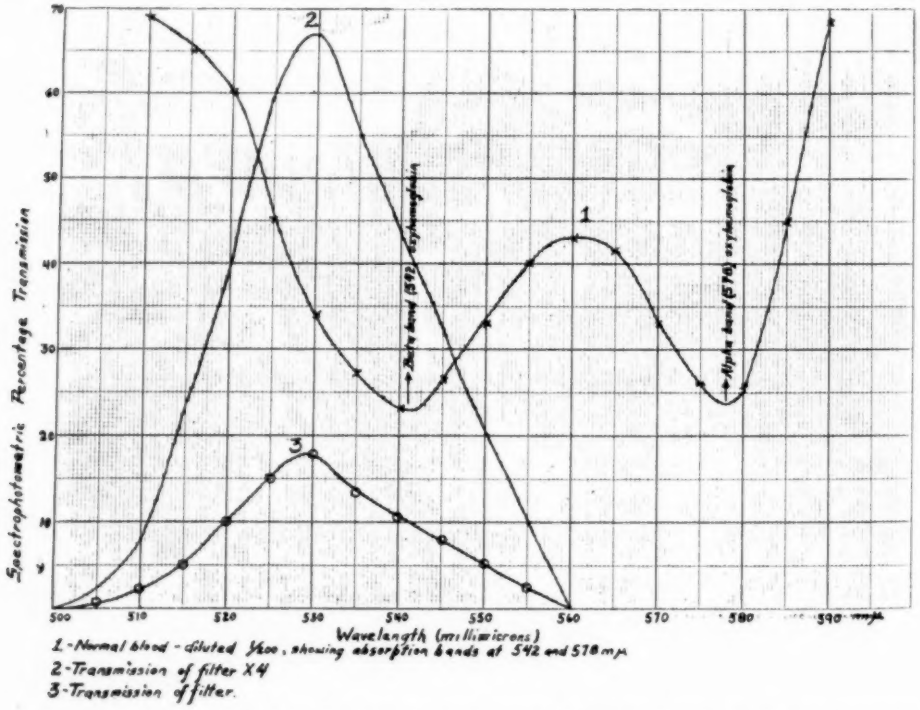

Fig. 4.—Spectrophotometric data showing the oxyhemoglobin bands (Curve 1), the transmission of the spectral filter (Curve 3) and the transmission of the filter × 4 (Curve 2).

center at 542 millimicrons. Curve 3 gives the spectrophotometric curve of light transmitted by the spectral filter which could be obtained commercially (and hence easily replaced) and which had the closest approximation to or was the counterpart of the absorption zone of the beta band of oxyhemoglobin. Curve 2 contains the data of Curve 3 multiplied by four. This has been done to show the extent of agreement (or disagreement) of the maximal point of transmission of the filter and the maximal point of absorption as shown in the beta band of oxyhemoglobin. The maximal transmission of the filter lies at 530 millimicrons; that of the beta band at 542 millimicrons.

The band of light transmitted by the filter is not commensurate wholly in shape or maximal point with the beta absorption band and to that extent may be the source of some discrepancy and the cause of part, if not all, of the percentage errors obtained in the use of the hemoglobinometer.

Spectrophotometry has the advantage that all readings on the spectral transmission of various samples of blood can be made monochromatically and at 542 millimicrons, for example. This was done by Williamson and again by Davis and Sheard.

The illumination passing through the sample of blood and the light filter and entering the photo-electric cell is, therefore, not monochromatic in character but is limited to a spectral region determined by the percentages of transmission of light by the blood and by the selective filter when taken together. Such percentages of light transmitted for various wave-lengths by the combined solution of blood and the filter can be calculated from the curves of Fig. 4. The spectrophotometric data on a certain sample of diluted (1:200) blood (having approximately 15 gm. of hemoglobin for each 100 c.c.) and the corresponding data on the combined transmission of the same sample of blood and the filter as experimentally determined are shown in Table I.

TABLE I

IN COLUMN B ARE SHOWN THE SPECTROPHOTOMETRIC READINGS ON A GIVEN SAMPLE OF BLOOD (DILUTION 1:200) AND IN COLUMN C THE COMBINED TRANSMISSION FOR BLOOD AND FILTER FOR THE WAVE-LENGTHS (IN MILLIMICRONS) GIVEN IN COLUMN A.

A	B	C
555	45	2.0
553		2.0
550	38	2.0
548		3.0
545	32	3.0
542		3.0
540	31	3.8
538		4.5
535	37	5.0
533		6.5
530	47	7.0
528		8.0
525	54	8.0
523		8.0
520	59	8.0
518		7.5
515	63	5.0
512		3.0
510	64	1.0

These experimental data show that the greater portion of the radiant energy transmitted by the combination of sample of blood and filter lies in the region of 540 to 515 millimicrons, with a maximal value at 530 to 520 millimicrons.

Theoretically, matters might be improved if one could use monochromatic radiation of a wave-length approximating the wave-length of 542 millimicrons. The green line of mercury fulfills these conditions, but the difficulties incurred in keeping a mercury vapor lamp constant in operation, and hence in illuminating power, and at the same time of getting sufficient energy to operate the

photo-electric cell without using a galvanometer of the highest sensitivity (such as a Coblentz) rather rule out this possibility.

The other mode of attack is to develop a filter which will more closely approximate the ideal. This we are endeavoring to do.

We again state that the combined use of spectral filter Eastman 74 and diluted blood gives us the closest practical approach to an illumination of the photo-electric photometer with monochromatic radiation and that the photo-electric photometer, under these circumstances, summates the energy of the incident light contained in a rather narrow spectral region.

THE RELATIONSHIP BETWEEN CONCENTRATIONS OF SOLUTIONS AND THE AMOUNTS OF TRANSMITTED LIGHT

The principles of spectrophotometry as applied to the determination of the amount of a substance in solution can be obtained from textbooks of physical chemistry, physiology, or in the literature in which applications of such principles have been made. Briefly, however, the symbol ϵ is used for the coefficient of extinction, so that if D_1 is the depth of solution required to reduce light of a given wave-length to one-tenth its value, then the extinction coefficient for this wave-length is

$$\epsilon = \frac{1}{D_1} \qquad (1)$$

In practice, it is the intensity (I) of the light which is measured. It is more convenient to use a constant thickness of solution and to measure the intensity of the light that has passed through this than to vary the thickness of the absorbing layer. This relationship is expressed in Lambert's law

$$\epsilon = \frac{-\log I}{D} \qquad (2)$$

If D is kept constant (say D = 1 cm., as in our work), then

$$\epsilon = -\log I \qquad (3)$$

From Beer's law, the absorption of light is directly proportional to the concentration; that is,

$$\frac{\epsilon_1}{\epsilon_2} = \frac{C_1}{C_2} \qquad (4)$$

It follows, therefore, that

$$\frac{\epsilon_1}{\epsilon_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} \qquad (5)$$

Hence, the concentrations (as, for example, relative amounts of hemoglobin) are directly proportional to the negative logarithms of the unabsorbed light.

If, therefore, the grams of hemoglobin per 100 c.c. of blood and the spectrophotometric reading for a given solution of blood for a given wave-length of light (say, 542 millimicrons) are known, then it is possible to calculate the grams of hemoglobin of an unknown sample from the combination of the laws of Lambert and Beer given in equation 5. Since successive dilution is equivalent to corresponding reductions in the amount of hemoglobin, it follows that if dilution is plotted against the logarithmic values of the

spectrophotometric readings, a linear relationship should be found to exist. This is shown to be the case in Curve 1 of Fig. 5. The percentage of accuracy is all that could be desired.

THE RELATIONSHIP BETWEEN CURRENT AND ILLUMINATION IN THE PHOTO-ELECTRIC PHOTOMETER

Fig. 6 demonstrates the fact that the illumination (I) received by the photo-electric cell is directly proportional to the current (A) set up in the

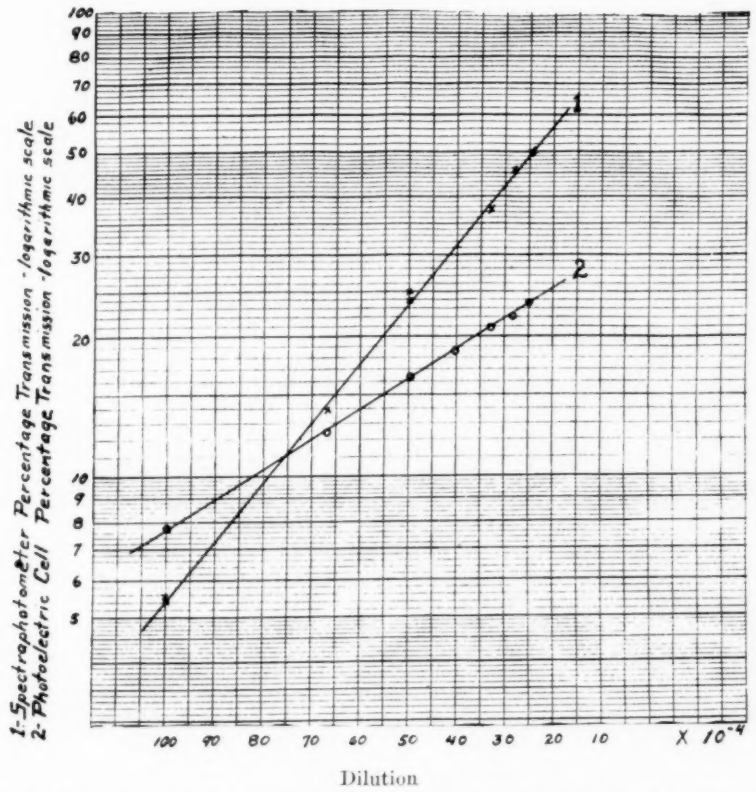

Fig. 5.—Curve 1. Showing the linear relationship between dilution of a given sample of blood and the spectrophotometric transmission at 542 millimicrons when plotted on semi-logarithmic paper. Curve 2, same relationship when the readings of the photo-electric cell, using the selective spectral filter, are plotted against dilution.

circuit containing the photo-electric cell and galvanometer provided the voltage remains a constant. Hence,

$$A = kI. \quad \text{(6)}$$

If the current in the lamp which serves as the source of illumination (Fig. 2, A) remains constant, then the current in the galvanometer in the photo-electric photometer will depend on the amount of light which is transmitted by the combination of sample of blood and spectral filter. Since the amount of light thus transmitted in the spectral region selected is dependent on the concentration (C) of hemoglobin in the sample of blood used and

since the laws governing concentrations of material in solutions and amounts of transmitted light indicate that

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2}$$

it follows that

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2} \quad - \quad - \quad - \quad (7)$$

This is shown to be the case experimentally in Curve 2 of Fig. 5, in which the same dilutions of blood as had previously been examined spectrophotometrically (Fig. 5, Curve 1) were tested with the photo-electric photometer.

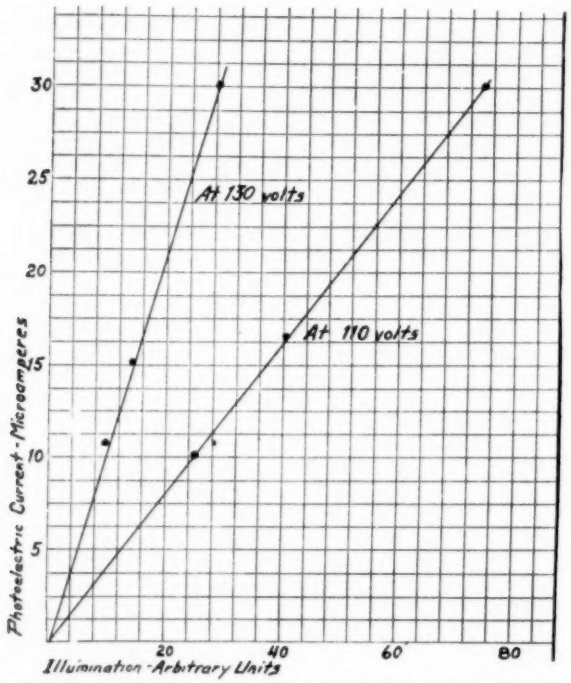

Fig. 6.—Curves showing that, for a given impressed voltage, the relationship between illumination and current (micro-amperes) in the circuit containing the photo-electric cell is linear.

CORRELATION BETWEEN GRAMS OF HEMOGLOBIN AS DETERMINED BY THE VAN SLYKE METHOD, THE SPECTROPHOTOMETER AND THE PHOTO-ELECTRIC HEMOGLOBINOMETER

The oxygen capacity method of van Slyke gives, by simple calculation and with a probable error of about 1 per cent, the grams of hemoglobin for each 100 c.c. of blood. Let the van Slyke reading be indicated by V. Then we have the correlation of grams of hemoglobin as determined by the van Slyke method, the spectrophotometer and the photo-electric hemoglobinometer given by the equation

$$\frac{V_1}{V_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2} \quad - \quad - \quad - \quad - \quad (8)$$

EXPERIMENTAL RESULTS

During the course of these investigations we have examined a large number of samples of blood at a constant dilution of 1:200 by the van Slyke, spectrophotometric and photo-electric photometric methods. Comparisons of experimental data with the results predicted by theory have formed a considerable portion of our experimentation for the reason that we were cogni-

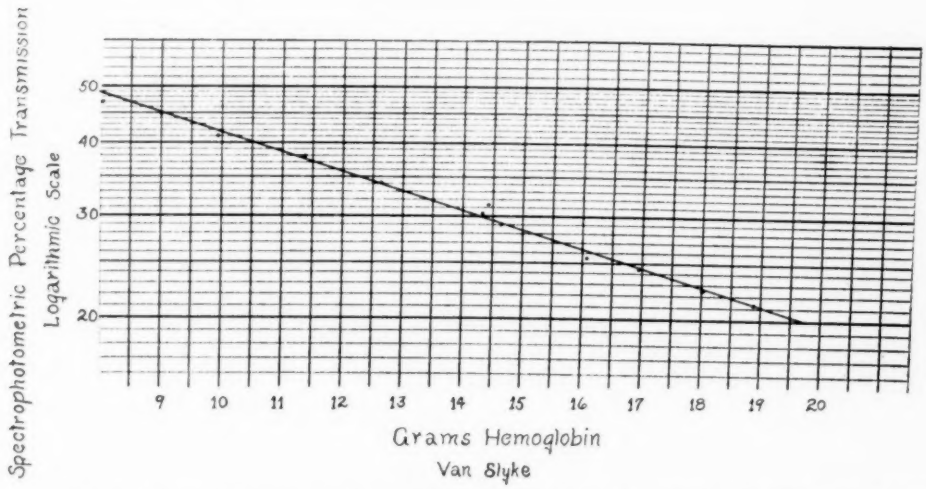

Fig. 7.—Curve showing that a linear relationship exists between grams hemoglobin, determined by the van Slyke method, and the spectrophotometric percentage transmissions plotted logarithmically.

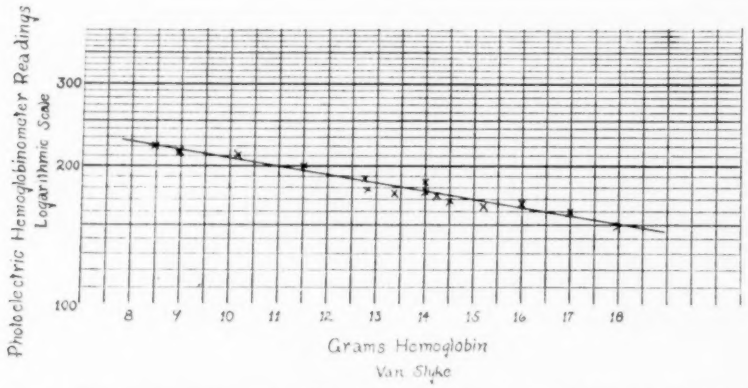

Fig. 8.—Curve showing the linear relationship between grams hemoglobin and the photo-electric hemoglobinometer readings plotted on a logarithmic scale.

zant of the possible sources of error in matters of dilution, readings on the spectrophotometer, inconstancy and apparent variability of the readings on the photo-electric photometer, lack of monochromatism of illumination in the photo-electric photometer and the like. Radical disagreement between theoretic and practical results led to consistent and careful checking and various attempts at minimizing or eliminating such errors. These attempts to cull out sources of error have resulted in the adoption of the following criteria:

1. Dilutions of the blood in the ratio of 1 part blood to 200 parts of diluent (0.5 per cent solution of sodium carbonate).

2. The adoption of an arbitrary or fiducial galvanometric deflection, making such adjustments of the iris diaphragm placed in front of the source of illumination as would give this arbitrarily chosen galvanometric reading when the absorption cell was removed from the instrument and the potentiometric device connected to the light source indicated a null reading on the milliammeter.

Figs. 7 to 10 inclusive contain data obtained by the three methods used in these investigations. The samples of blood used were selected so as to cover

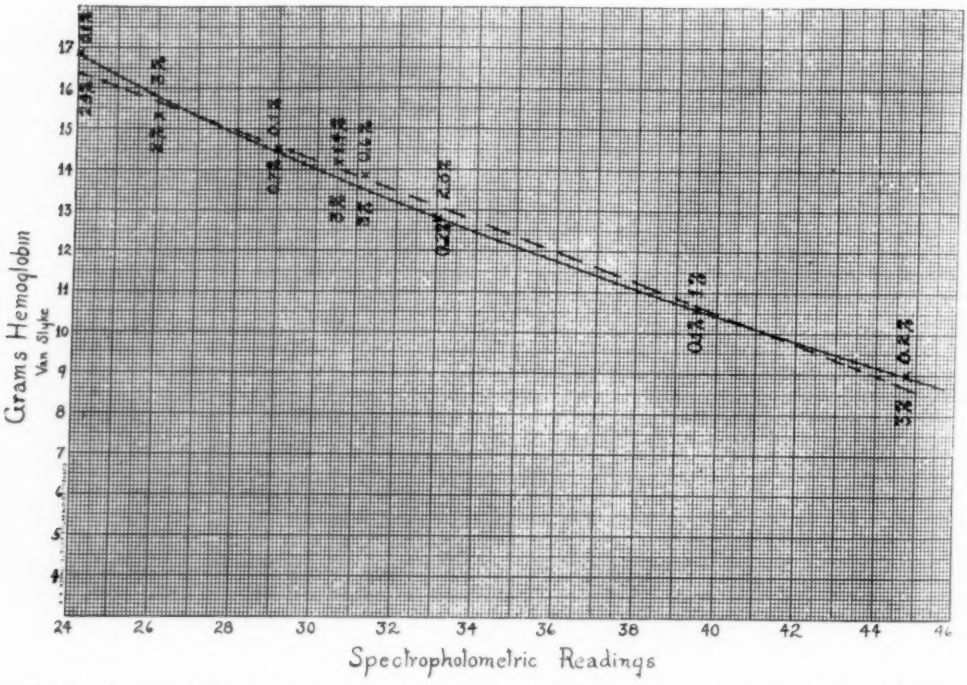

Fig. 9.—Curves showing the experimentally determined relation between grams of hemoglobin and the spectrophotometric readings for various samples of blood (dilution 1:200).

a reasonably wide range of values of hemoglobin. In the results shown in Figs. 7 to 10, preparation and dilution of the blood samples was made by a technician in the laboratories, the readings on the photo-hemoglobinometer were also made by a technical helper in the section of physics. The van Slyke and spectrophotometric readings were made by persons experienced in these fields. Therefore, it is to be presumed that the van Slyke values (which are taken as the standard) and the spectrophotometric readings are as accurate as are likely to be obtained. The routine dilution of blood and the matter of readings on the photo-electric photometer, on the other hand, approximated ordinary working conditions which should improve in accuracy as those who work with them improve in skill. As a matter of fact, however, an inspection of the results obtained by either the spectrophotometer or the photo-electric

hemoglobinometer, when compared with the values on the van Slyke apparatus, shows that the percentage error by either method is not in excess of 4 per cent and averages less than 2 per cent for the group of readings shown (if such an average is justifiable and means anything).

The test of the accuracy of either spectrophotometry or photo-electric photometry as applied to the determination of amounts of hemoglobin lies in equation (8), that is,

$$\frac{V_1}{V_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2}$$

The relationship between concentration (van Slyke) and amount or percentage of light transmitted in a given spectral region by various concentrations

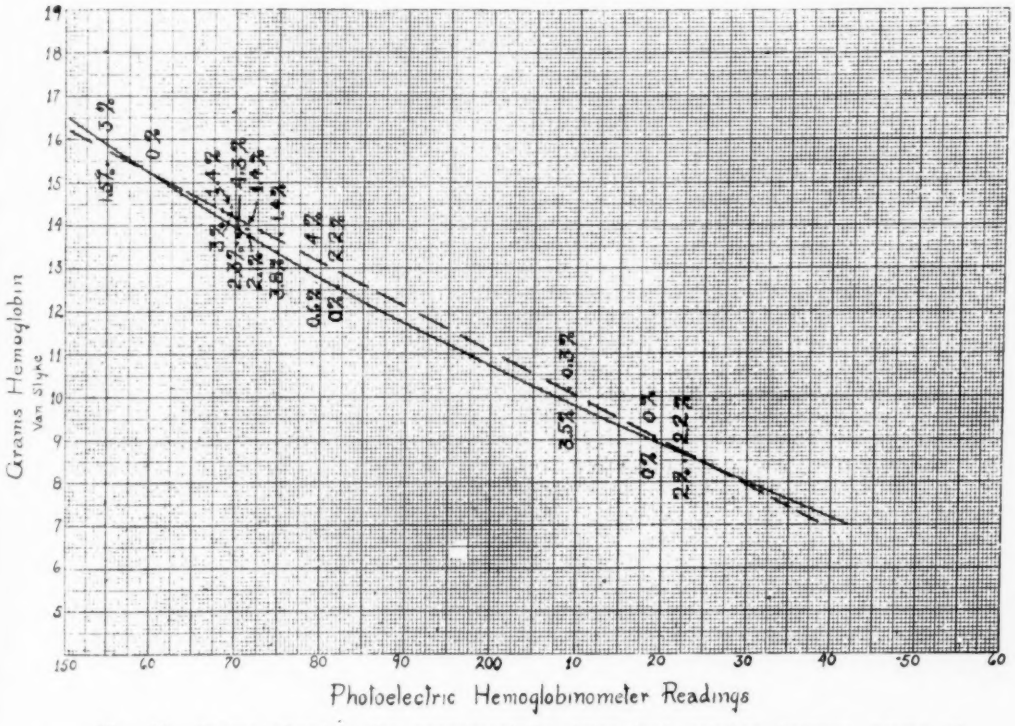

Fig. 10.—Curves showing the experimentally determined relation between grams of hemoglobin and the readings of the photo-electric hemoglobinometer for various samples of blood (dilution 1/200).

of hemoglobin is such that all points plotted should lie on a straight line when concentrations are plotted against the logarithmic values of the transmitted light. Figs. 7 and 8 show how closely theory and practical results agree.

For routine work, however, it would appear to be more advantageous to make charts in which logarithms were eliminated. Figs. 9 and 10, therefore, contain the values determined spectrophotometrically and with the photo-electric hemoglobinometer, plotted as abscissae and the determinations of grams of hemoglobin by the van Slyke method as ordinates. In drawing the curves, no regard for theoretic considerations has maintained but an attempt

has been made to draw a curve (or curves) which would include the largest number of points. The slightly curved lines in both charts more closely approximate the theoretic form of curve, although it is possible to make determinations of grams hemoglobin within the range of the chart (17 to 8 gm.) if straight lines are drawn through the largest number of points possible. To make the final chart and to draw in the nearest theoretically correct and most applicable experimental curve requires the accumulation of a considerable amount of data, particularly covering the additional ranges of polycythemia and severe anemias.

In the case of the curves of Figs. 9 and 10, we have indicated the percentages of error, taking the van Slyke values as standards, with reference to both the curved and straight lines drawn in the diagrams. These two charts (Figs. 9 and 10) constitute a fair representation of the accuracy of the spectrophotometer and of the photometric hemoglobinometer within a range of 17 to 8 gm. of hemoglobin.

TABLE II

COMPARISON OF DATA ON THE DETERMINATION OF HEMOGLOBIN BY VARIOUS METHODS

NO.	SEX	AGE	RED BLOOD CELLS, MILLIONS	DARE HB	GRAMS, HEMOGLOBIN PER 100 C.C.			PERCENTAGE ERROR IN PHOTO-ELECTRIC HEMOGLOBINOMETER	
					VAN SLYKE	SPECTRO-PHOTOMETER	PHOTO-ELECTRIC	VAN SLYKE	SPECTRO-PHOTOMETER
1	M	46	5.02	87	16.91	16.80	16.60	−1.8	−1.3
2	M	33	4.75	76	15.35	15.60	15.70	+2.3	+0.6
3	F	22	4.80	77	15.21	15.70	15.20	0	−3.1
4	F	31	4.93	78	----	15.30	15.70	----	+2.6
5	F	30	----	--	----	15.10	15.00	----	−0.6
6	F	21	4.46	71	----	14.60	14.20	----	−2.8
7	F	70	4.52	72	14.46	14.50	14.20	−2.0	−2.0
8	F	39	4.92	80	14.30	14.00	14.50	+1.3	+3.6
9	F	51	4.37	69	14.02	14.00	13.40	−4.3	−4.3
10	F	25	----	80	13.96	13.70	13.90	−0.7	+1.4
11	F	42	3.73	63	----	13.80	13.20	----	−4.3
12	F	52	4.10	73	----	13.50	13.00	----	−3.6
13	F	51	4.26	71	13.41	----	14.00	+4.5	----
14	F	67	4.15	75	----	14.00	13.30	----	−5.0
15	F	70	3.89	68	12.72	12.70	12.90	+1.5	+1.5
16	F	62	4.28	63	12.68	----	12.70	0	----
17	F	36	4.00	59	10.15	10.40	9.90	−2.5	−4.8
18	F	35	3.86	55	----	10.10	9.70	----	−4.0
19	F	21	3.82	45	9.02	8.80	9.00	0	+2.2
20	F	52	3.17	33	8.56	----	8.50	0	----
21	M	65	3.47	36	----	7.50	7.80	----	+4.0
22	F	57	3.08	39	6.71	10.10	9.90	----	−2.0

Table II contains collected data regarding the sex, age, red blood cell count, Dare reading, van Slyke, spectrophotometric and photo-electric photometric readings, and percentages of error in a group of routine cases during the two weeks prior to the preparation of this paper.

SUMMARY

An outline of the data we have presented in this paper is as follows:

1. A brief discussion of various methods of determining the percentage or grams of hemoglobin and their sources of error.

2. A description of a new type of instrument, the photo-electric hemoglobinometer, for the accurate and rapid determination of hemoglobin expressed in grams for each 100 c.c. of blood.

3. Complete details regarding the modus operandi of the photo-electric hemoglobinometer.

4. The theoretical considerations involved in photo-electric photometry as applied to determinations of hemoglobin, indicating that the readings on this instrument should follow the laws of Lambert and Beer.

5. Experimental data obtained by the van Slyke, spectrophotometer and photo-electric hemoglobinometer and their comparison.

6. Data substantiating the belief that the photo-electric hemoglobinometer will permit of the determination of grams of hemoglobin within less than 5 per cent of the van Slyke determinations, which have been used as standards.

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DISCUSSION

Dr. Wm. G. Exton.—May I say that I have never presented a hemoglobin method. I have presented a general method of colorimetry which is applicable to many things besides hemoglobin. Its applicability to hemoglobin is demonstrated of course by the fact that Dr. Sheard employs my method of colorimetry in his method of determining hemoglobin. Dr. Sheard commented on the straightness of the lines in the charts, which is somewhat characteristic of the extinction method.

I want to say that I think Dr. Sheard's method is particularly beautiful. My extinction method has the defect of varying with different observers, and this defect Dr. Sheard has overcome by employing with it what he calls the electric eye. Certainly nothing could be prettier in theory.

Dr. F. E. Sondern.—Dr. Exton has promised us considerable relief and while I hope to live to see this I must say my patience is beginning to wear. I hope we will not have the same experience with this new apparatus. If you can give us some sort of idea as to how much patience we will have to have we would appreciate it.

Dr. Charles Sheard.—The apparatus is still in the experimental stage. The model which we have used in obtaining the results contained in this paper can be improved upon, and we are hoping to do this in the near future. If any patent rights are granted they will be assigned to this Society. The only reason for desiring such patent rights is that we want instruments, when they are placed upon the market, to be as accurate and serviceable as possible. We hope that we may have a satisfactory instrument, suitable for use in any laboratory, ready within the next year.

Dr. Wm. G. Exton.—If Dr. Sheard's experience is at all like mine, I am sorry to have to tell him that when he begins with the apparatus makers he will find his troubles have just begun.

Dr. A. H. Sanford.—Dr. Sondern has had experience with physicists before. Dr. Sheard has proved to himself that he is right. I hope that it will be practical to make standard instruments that can be used in institutions.

Dr. Alvin G. Foord.—May I ask if anyone has used oxalated venous blood? There is a slight difference in capillary or venous blood in the hemoglobin value.

Dr. A. H. Sanford.—We tried oxalated blood. The trouble with making dilutions of oxalated blood was that there seemed to be a sedimentation factor that entered in. It would be possible I believe to use oxalated blood if you were willing to take into consideration the slight error due to sedimentation. We used whole blood drawn from the vein and made the dilutions at once using a 0.2 c.c. pipette graduated in thousandths. Draw the blood to the 0.1 c.c. mark, wipe off the tip, dilute in 20 c.c. of sodium carbonate to make a good clear oxyhemoglobin solution free from fibrin precipitate.

OBITUARIES

REPORT OF THE COMMITTEE ON NECROLOGY

FATE has been very unkind to the American Society of Clinical Pathologists in robbing us, within the past year, of six men who had been valuable members of our organization and whose absence from our ranks will be greatly felt. It is not alone the comparatively large proportion of death loss that we deplore, but the high quality of the men who were snatched from our midst by the Grim Reaper and which gives us greater cause for sorrow.

The mournful news of the death of our Vice-President, Lieut. Colonel H. J. Nichols was made more poignant a few days later by the untimely demise of a promising young member, Dr. Dean N. Beacom of Denver. Another sad demise was that of young Dr. Reed Rockwood of Baltimore. In January of this year we lost the beloved author of the most popular textbook on *Clinical Pathology*, Dr. James C. Todd, of Boulder.

The greatest blow to the Society was the sudden death of our valuable secretary and cofounder of the American Society of Clinical Pathologists, Dr. Ward Burdick. His spirit still hovers over our meeting and hardly an official action is taken but what our minds revert to the memory of him who by his gentle presence elicited the love and esteem of all his fellow members.

It is also our sad lot to record the passing of Dr. Charles E. Simon, who through his textbook on *Clinical Pathology* started many of our members on their successful careers in this specialty.

The Committee presents herewith an obituary and some biographical data of each of our departed fellow workers and requests that suitable resolutions of condolence be sent to their respective families.

COMMITTEE ON NECROLOGY
PHILIP HILLKOWITZ, *Chairman*
FRANK W. HARTMAN
ROBERT A. KEILTY
CHARLES C. W. JUDD

LIEUTENANT COLONEL HENRY J. NICHOLS

LIEUTENANT COLONEL HENRY J. NICHOLS died at Panama, September 3, 1927, after an operation for appendicitis. He was born in Milwaukee, Wisconsin, May 21, 1877, and appointed in the Medical Corps of the Army from New York, June 20, 1906. He was B.A. '99 and A.M. '01 of Yale and graduated in medicine at the University of Pennsylvania in 1904. After passing through the lower grades he became Lieutenant Colonel June 30, 1926, having also held this grade during the World War.

Colonel Nichols was one of the most distinguished scientific men in the Medical Corps. He was Assistant Director of Laboratories of the Army Medi-

LIEUTENANT COLONEL HENRY J. NICHOLS

cal School, 1911-1914, and Director, 1923-1926. His name is associated with that of Russell in the development and perfection of typhoid immunization. He was an eminent syphilologist and was a pioneer in the introduction of the use of salvarsan in America. He had been President of the American Society of Tropical Medicine and was Editor of its Journal up to the time of his departure for Panama, where he was, at the time of his death, Medical Inspector of the Department.

He was the author of the work, *Carriers in Infectious Diseases,* and of many valuable contributions to periodical medical literature. He was a member of the Phi Beta Kappa fraternity.

Dr. Nichols was a Charter Member of the American Society of Clinical Pathologists, and at the time of his death was Vice-President of this organization.

His loss is greatly mourned by our Fellows.

DEAN NOLON BEACOM, M.D.

DR. DEAN N. BEACOM died suddenly at his home in Denver, September 6, 1927, from a pulmonary hemorrhage. He had been in apparent good health and his sudden demise came as a shock to his many friends and associates.

Dr. Beacom was born in Blandisville, Illinois, November 1, 1894. He attended the various public schools in that region, and finally graduated from the Western Illinois State Teachers College.

Shortly after this he came to Colorado for his health. Making his residence at Boulder he attended the University of Colorado, first in the Liberal Arts College, from which he received the degree of Bachelor of Arts, and in

DEAN NOLON BEACOM, M.D.

June, 1922, he graduated from the School of Medicine. The following year he spent in the Agnes Memorial Sanitarium. In September, 1923, he became associated with the Department of Clinical Pathology of the University of Colorado, moving to Denver with the opening of the new buildings of the School of Medicine in September, 1924, and had continued in this position to the day of his death.

He belonged to the Medical Society of the City and County of Denver, the Colorado State Medical Society, was a Fellow of the American Society of Clinical Pathologists, and was Secretary of the Colorado Society of Clinical Pathologists. He also belonged to the Alpha Tau Omega and Phi Rho Sigma fraternities, and was a Mason.

His death is greatly mourned by our members.

—*E. R. M.*

CHARLES EDMUND SIMON, M.D.

DR. CHARLES EDMUND SIMON was a Charter Member of the American Society of Clinical Pathologists and at the time of his death held the professorial chair of the Department of Filtrable Viruses at the Johns Hopkins School of Hygiene and Public Health.

He was born in Baltimore, Maryland, September 23, 1866, and died at his home in Baltimore, November 8, 1927. He came of substantial Teutonic stock. His parents considered that education in this country was inferior to the German system. In consequence his entire precollege education was obtained in Germany. He studied in both Baden-Baden and in Hanover.

The opening of the Johns Hopkins University in his home city attracted him there in his eighteenth year. He took his B.A. in 1888, majoring in the sciences and particularly enjoying the work of Martin's classes. He began his medical course at the University of Pennsylvania. There he attracted the attention of that keen seeker of talent, Wm. Osler. Arrangements were made for the completion of the prescribed medical curriculum at the University of Maryland, where Simon's degree of M.D. was granted in 1890, while simultaneously he served Dr. Osler on his Medical Service at the then newly opened Johns Hopkins Hospital. Here his opportunities were varied. He worked in the neurologic dispensary. He worked on the wards. But most of all he was attracted by the laboratory elucidation of diseased states which he encountered. Osler was quick to appreciate this and acting upon his advice Simon spent a year or more in Paris under Gautier and in Basel under Bunge. It is probable that the hope of an appointment to the teaching staff of the Johns Hopkins Medical School was entertained by Dr. Simon, but he returned from abroad with his Swiss bride to assume duties for which provision was not made.

Disappointed in this expectation, he renounced a teaching career and entered private practice. For this he had little stomach, though he soon built up an active following and did much good work as a gastroenterologist. The lure of teaching was strong, however, and he drifted back to Hopkins and worked faithfully there in the laboratories. All this time he was working indefatigably at all odd moments on the production of his text, *Clinical Diagnosis*. The first edition of this appeared in 1896. In the quarter century following, nine subsequent editions made their appearance. Each presented all important new developments in the science of clinical pathology. In clarity of expression, in arrangement of subject matter and explicitness of directions it has few competitors. It is a standard text in every sense of the word.

The ten years following the appearance of the first edition of this work was a decade of discouragement and ill health, during which he led a most sequestered life. The development of fixed fears unfitted him for carrying the burdens of his private practice. His work at Hopkins was abandoned. He was forced to live deprived of all normal human contacts. The summer

days in the open or on a boat on Nova Scotia waters brought back some health, but the weary months of winter again would sap his little store of vigor. The unproductiveness of this period weighed upon him. It was Dr. Osler, the student of men as well as medicine, who solved the problem. He suggested that an old stable on the property occupied by Dr. Simon be converted into a diagnostic laboratory. Note the sapiency of that suggestion. The element of hope, the element of occupational therapy, the element of the pioneer were all rolled into one. It was planted in the soul of one naturally imbued with enthusiasm, and the will to do great things. This began what was probably the first private venture of the sort attempted in this country. In this laboratory he and his wife spent much time, doing not only the ever increasing volume of work submitted to him by local and distant physicians, but also investigating problems of more purely scientific value. Numerous contributions on many topics emanated from this humble stable. Here, too, he won for himself a country-wide recognition in this field of endeavor. Most of all he began to come out of his state of retirement and seclusion.

The old fever of the teacher fired him. At first it was a chosen few earnest students, who, wishing to perfect themselves in clinical pathology and establish laboratories elsewhere, came to learn first hand from this pioneer. Later he accepted invitations to join the staffs of several local hospitals, organizing their laboratories and stimulating their house staffs to greater endeavor. Finally in about 1910 he burst forth in full swing as a teacher. He became Professor of Clinical Pathology at the College of Physicians and Surgeons, of Baltimore. In 1915 with the merger of this school with the University of Maryland, his medical Alma Mater, he assumed charge of the department in the combined schools. His enthusiasm in the work, his interest in the individual pupil and his assistants, together with his masterly comprehension of his subject, made him a teacher of rare quality.

In addition to this teaching, he invariably had some laboratory research under way, the results of which he (alone or often generously including some of his assistants) published in the current literature. Not satisfied with the scope of his first text publication, following the success of Paul Th. Muller's book in Germany, he published the first comprehensive text in this country on *Infection and Immunity* in 1912. This was followed by several other editions. Still further afield, there appeared in 1919 *Human Infection Carriers.* In the same year he epitomized his clear-cut and mature ideas of how to present the subject of Clinical Pathology to the undergraduate student in a small *Laboratory Syllabus of Clinical Pathology.*

The last period of Dr. Simon's life, though marked by a transition from functional to organic bodily handicap, was, none the less, one of deep satisfaction to him and contained more of the joy of living than any previous decade. The Johns Hopkins School of Hygiene and Public Health was started in 1918. Simon soon became intensely interested in its work. His wide clinical knowledge and his training under Martin made him a valuable advisor in the correlation of pure zoology with medicine. In 1920 he began lecturing on medical zoology. Early in the development of the school Dr. Welch had planned for a western world publication on hygiene. Simon's experience in

the preparation of texts for publication suggested his fitness for the business managership of this enterprise. In 1921 Simon undertook the task of launching the *American Journal of Hygiene* with his inimitable enthusiasm. Seven volumes of this periodical were published under his discriminating management.

Along with this work he undertook the serious investigation of the rôle of the filtrable viruses in both animal and plant pathology. He collected a practically complete literature on the subject, he lectured and later conducted a laboratory course with the students, he trained new men in this field, he experimented and investigated and he got in touch by letter and personal conference with nearly all investigators on the subject both in this country and in Europe. The development of this twilight zone between chemistry and zoology received such impetus under his guidance, that in 1927 the institution established a separate department and created the first chair of the subject in any university of the United States. Dr. Simon was appointed to this professorial chair.

Imbued as he was with the German idea of scholastic attainment, this appointment in a congenial, stimulating atmosphere of learning was the crownpoint of his career. His life of endeavor against odds was at last a success in his own eyes, and there were none more critical. His cup of joy was filled. This appointment was to Simon what the vision of The Child was to Simeon. It was the enunciation of Nunc Dimittis.

As a student and a seeker of truth, as investigator of the secrets of science, as writer, editor and above all as a teacher, his death is a universal loss. To those fortunate few who succeeded in knowing him well, his life was an inspiration and incentive to carry on the flame of knowledge and his death is a personal loss.

It is with profound regret that the American Society of Clinical Pathologists records the death of this eminent member.

—*C. C. W. J.*

PAUL REED ROCKWOOD, M.D.

DR. PAUL REED ROCKWOOD was born in Iowa City, in 1897, and died in December, 1927.

He received his early education at the Morgan Park Military Academy, and his B.A. and M.D. degrees from the University of Iowa, the last named in 1922. After serving an internship in the University Hospital he became a Fellow of the Mayo Clinic where he remained for three years. At the time of his death he was Assistant Professor of Medicine in the Medical School of the University of Maryland, at Baltimore.

His early death is deeply deplored.

—*P. H.*

JAMES CAMPBELL TODD, M.D.

JAMES CAMPBELL TODD died at his home in Boulder, Colorado, the evening of January 6, 1928, following a long illness.

Born in Shreve, Ohio, March 17, 1874, he graduated from Wooster College in 1897, with a degree of Bachelor of Philosophy. He continued his studies in the University of Pennsylvania School of Medicine, from which he received the degree of M.D. in 1900.

While in Wooster College he held the position of Assistant in Biology during 1895-96. From 1900-01 he was resident physician in the Allegheny

JAMES CAMPBELL TODD, M.D.

General Hospital, Pittsburgh. About this time his health failed, and he moved to Colorado, where he located in Denver.

He soon became identified in the field of medical education, first as Assistant in Pathology in the Denver and Gross College of Medicine during 1904-05, then as lecturer from 1905-08, later as Associate Professor from 1908-10, and assumed the professorship of the department in 1910.

On January 1, 1911, the University of Colorado School of Medicine absorbed the Denver and Gross College of Medicine, the two faculties were merged, and Dr. Todd became Professor of Pathology in the Boulder division. He also acted as the secretary of this division until 1916. Since 1923 he has been premedical advisor in the university.

As the study of pathology broadened he felt that he was losing the con-

tact in the fields of hematology and parasitology he desired. So in 1916 he became Professor and head of the Department of Clinical Pathology which had just been created at his request. These positions he held at the time of his death.

During these years ill health became an increasing handicap, but in the face of these difficulties he showed a determination and persistency of purpose that won the admiration of both students and faculty. His enthusiasm for his work was transmitted to all who had the pleasure to work with him. He was ever willing to aid, and considered the rôle of a teacher as his highest ambition.

Early in his teaching career he found that little or nothing had been compiled in clinical laboratory methods of value to the general practitioner. The collecting and testing of such laboratory procedures became his life work. He kept the viewpoint of the average medical man before him, and simplicity of technic as well as the accuracy of results claimed his closest attention.

His book, *Clinical Diagnosis by Laboratory Methods,* first appeared in 1908, and in the different editions he placed all his writings, with the exception of a few early articles. This book has become established over the world as an authority in its field, and it has with few exceptions enjoyed as widespread a distribution as any medical book published in English. He was sole author of the first five editions. The sixth edition, which came out in September, 1927, was written in collaboration with Dr. Arthur Hawley Sanford of the Mayo Clinic. His determination and perseverance may be realized by the fact that the work on all editions of his book was done while bedfast or in a rocking chair.

In recognition of his ability in this field Dr. Todd was the recipient of many honors. Modest to the point of bashfulness, he would seldom speak of his own work except in a reticent manner; but his opinions on diagnostic methods always demanded respect, for they were given only after due consideration, and then in a decisive manner.

He became a Fellow of the American College of Physicians in 1922, was a member of Sigma Xi, and was given honorary membership in Phi Beta Kappa by his Alma Mater in recognition of his ability and learning. He was a Fellow and Charter Member of the American Society of Clinical Pathologists, a Fellow of the American Association for the Advancement of Science, a Fellow of the American Medical Association and an honorary member of the Colorado Society of Clinical Pathologists.

Four years ago he was compelled to give up active teaching, but kept in close touch with the affairs of his department, the university, and the world. He read much, not only in his particular field, but on general subjects. He will be missed in particular by his old students, and by faculty members of the university, who enjoyed his ability as a conversationalist, who admired him for his accomplishments and respected his ability and scholarship. His memory will be cherished as that of a man whom all could well emulate.

It may be interesting to add that Dr. Todd was the first to advocate a Chair of Clinical Pathology in Medical Schools.

—*E. R. M.*

WARD THOMAS BURDICK, M.D.

THE American Society of Clinical Pathologists has sustained a severe loss in the untimely death of Dr. Ward Burdick. In his many years of service to the medical profession in the capacity of clinical pathologist he endeared himself to all who came in contact with him and none could help but admire his gentleness of character and amiability of disposition.

Dr. Ward Thomas Burdick was born April 5, 1880, in McKane county, Pennsylvania. He graduated from the National University of Arts and Sciences, in St. Louis, Mo., in 1908, and located in Colorado in 1911. Soon after

WARD THOMAS BURDICK, M.D.

his arrival he became interested in clinical pathology and devoted the rest of his life to this branch of medicine. He served successively as laboratory director in the National Jewish Hospital, in the Denver General Hospital, and Children's Hospital. In all these positions, he earned the esteem of his colleagues by his ability, industry, and conscientiousness. He has rendered valuable services by the papers that he contributed on the technic of the Wassermann reaction and other scientific essays.

Dr. Burdick's share in the furtherance of scientific medicine of the country is signalized in the formation of the American Society of Clinical Pathologists which he helped to found and of which he was the national secretary from its inception until his death.

Dr. Burdick was distinguished by an extraordinarily fine character. He was a stickler for scientific accuracy and precision in technic as is evidenced

by the appearance of his laboratories which were models of orderliness and neatness. His passing will be greatly mourned both by the medical profession at large as well as by his numerous friends in the field of clinical pathology, throughout the United States, all of whom entertain for him a deep sense of esteem and affection.

His endeavors for the success of the organization will always serve as a stimulus and example to our younger colleagues.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

BACTERIA: A Practical Method for Single Cell Culture, Paine, S. G. J. Bact. 14: 441, 1927.

The method is a modification of the old method of separation by attenuation. The attenuations are made in sterile water in a series of five sterile watchglasses. A plate of nutrient agar with the bottom of the dish marked by Chinese ink with nine rows of about 10 spots in each row, is placed near by. The suspensions are then examined by placing a loopful of each turn under a cover-slip and viewing with the microscope to see that the organisms are not clumped and to form an opinion as to the density of the suspensions. From this inspection, with a little experience, one can judge which are the appropriate suspensions to employ. With a steel mapping pen, sterilized by alcohol and flaming, the weakest of three selected suspensions is taken and spotted on the surface of the agar to cover three of the marked rows, one drop being placed near to, but not quite exactly over, each of the ink spots. By just lightly touching the agar the droplet can be made so small that it will easily come within the field of a 2/3 objective, and usually one filling of the pen is sufficient to cover the whole of the thirty spots in the three rows.

The plates after inoculation are incubated overnight at a temperature suitable for the organism, and are examined with the low power of the microscope; the disk being placed bottom up, the position of each colony is found by searching the surface of the agar in the neighborhood of the ink spot on the bottom of the dish. If the plating has been satisfactorily carried out one finds that each spot, representing the strongest of the chosen suspensions, shows many initial minute colonies, say fifteen to twenty, which will later coalesce to form one colony. It seems quite clear under the microscope that most of these initial spots arise from a single cell. Passing down the three rows representing the second of the chosen suspensions one finds the initial spots rduced to a number of one to four with here and there a blank. Then the rows representing the greatest dilution will contain for the most part blanks with here and there a colony with one point of origin or possibly two points but no more. Now it is easy to see that all these colonies which one marks down as having arisen from one point, develop during the next few hours into colonies of precisely the same shape and size. Any in which one detects two points develop into colonies of greater size. One therefore feels that by selecting colonies marked as having one point of origin and neglecting any of these which subsequently develop to more than minimal size, one can confidently rely on such colonies having developed from single cells.

TISSUE: A Simple Method for Staining Reticulum, Kinney, E. M. Arch. Path. & Lab. Med. 5: No. 2, 284, 1928.

The solution, which simultaneously fixes the tissue and stains the block for reticulum, is prepared by adding 1 gm. of sodium sulphantimonate to 100 c.c. of 4 per cent formaldehyde immediately before using. The tissue should be cut only a few millimeters thick and for the best results should be fresh, although material which has been removed at autopsy several hours after death has been successfully stained by this method.

When this 1 per cent solution is to be used, the tissue should be removed after about eighteen hours. The fixing time may be shortened by from nine to twelve hours if a 1.5 per cent solution is used. In either case, however, the tissue should be removed at the end of the stated time, as the stain then seems to begin to disappear. The tissue is then treated as any other material which is to be embedded in paraffin,

with the exception that it cannot be left in the clearing agent more than from one to two hours, whether xylene or cedar oil is used. The stain completely disappears if the material is left in cedar oil for as long as twelve hours. The stain is stabile in alcohol, and the tissue may remain in 70 per cent alcohol as long as desired.

After the sections are cut and mounted in the usual manner, the paraffin is removed with xylene and the cover-slip immediately attached with neutral balsam.

By this method the reticulum is stained a dark brown and other connective tissue, yellow. An occasional nucleus may be stained. The remainder of the tissue is unaffected by this method and may be stained for routine observation by any of the ordinary stains, such as hematoxylin and eosin. However, hematoxylin or other basic stains obscure the reticulum.

RENAL FUNCTION: Influence of Posture on Phenolsulphonephthalein Test for Kidney Function, Cordero, N., and Freedman, M. H. Arch. Int. Med. **41**: No. 2, 279, 1928.

The elimination of phenolsulphonephthalein in normal persons is greater in the recumbent posture than during standing. The difference (from 10 to 13 per cent) though definite is not marked, but should be taken into account in making phenolsulphonephthalein tests in borderline cases. The possibility that the difference might be greater in cardiorenal disorders is pointed out.

The effect of recumbency in increasing the volume of output is marked (from 50 to 200 per cent), confirming the results of the earlier investigators.

The probable causes of these postural effects are discussed.

On the basis of fifty-seven experiments on two subjects, at intervals of at least five days, it is shown that the hypodermic route is not as unreliable as is generally believed when the two hour eliminations are taken into account.

The abscence of toxic effects during the eight months covered in these studies is offered as one more evidence of the harmlessness of phenolsulphonephthalein. There is no evidence of the development of any increased storage or increased destruction in the body during this frequent and long-continued use.

Slight modifications in the technic of renal function tests are suggested, namely, as a result of the foregoing studies and the possibilities mentioned, it seems essential that renal function tests should be made with the patients in recumbent posture to eliminate one more variable, especially in borderline cases. It might be advisable, furthermore, to give water in fractional doses, with a view to assuring at the time of collection of the sample, a volume large enough to stimulate micturition even in nervous subjects, and yet small enough to exclude the possibility of renal inhibition by bladder distension.

MILK: Use of a Differential Stain in the Direct Enumeration of Bacteria in Pasteurized Milk, Beattie, M. Am. J. Pub. Health **17**: 1031, 1927.

Dead bacteria, stained previously by Loeffler's blue, lose the blue color and become red under the influence of a 10 per cent solution of carbol-fuchsin poured rapidly over the preparation. Treated by the same process, the bacteria of a living culture do not give up the blue dye which they have fixed. The variable cyanophilia of bacteria, corresponding to their vitality, appears in a more certain manner if the following mixture is used to stain preparations fixed by moderate heat:

Concentrated fuchsin (Ziehl)	8 c.c.
Distilled water	100 c.c.
Loeffler's	100 c.c.

The coloring mixture ought to remain at least twenty-four hours exposed to the air before being used. It is sufficient to stain during one minute and to wash in water in order for the differential color to appear; blue for the living bacteria and red for those which are dead.

STAINS: Stain Solubilities, Holmes, W. C. Stain Technology 2: No. 3, 68, 1927.

The values reported are expressed as grams of anhydrous dye per hundred cubic centimeters of saturated solution. Bracketed values are expressed as grams of anhydrous dye per hundred grams of saturated solution.

DYE SOLUBILITIES AT 26° C.

	Water	95 *Per Cent Alcohol*
Martius Yellow (calcium salt); C. I. No. 9	0.05	1.90
Sudan I; C. I. No. 24	Nil	0.37
Sudan II; C. I. No. 73	Nil	0.39
Crystal Ponceau; C. I. No. 89	0.80	0.06
Orange IV; C. I. No. 143	0.16	0.20
Resorcin Yellow; C. I. No. 148	0.37	0.19
Sudan III; C. I. No. 248	Nil	0.15
Sudan IV; C. I. No. 258	Nil	0.09
Pararosanilin (acetate); C. I. 676	4.15	13.63
New Fuchsin (chloride); C. I. No. 678	1.13	3.20
New Fuchsin (chloride), Resorcin Addition Product*	0.94	5.74
Methyl Violet; C. I. No. 680	2.93	(15.21)
Crystal Violet (chloride); C. I. No. 681	1.68	13.87
Crystal Violet (iodide)	0.035	1.78
Crystal Violet (chloride), Resorcin Addition Product	0.28	13.84
Crystal Violet (chloride), Hydroquinone Addition Product	0.30	8.39
Crystal Violet (chloride), o Pyrocatechin Addition Product	0.79	24.87
Eosin; C. I. No. 768	44.20	2.18
Eosin B; C. I. No. 771	39.11	0.75
Erythrosin; C. I. No. 773	11.10	1.87
Methylene Blue (chloride); C. I. No. 922	3.55	1.48
Methylene Blue (iodide)	0.09	0.13
New Methylene Blue N; C. I. No. 927	(13.32)	1.65

*The various addition products of basic dyes and phenols listed are excellent stains for "acid-fast" organisms and spores which resist staining with basic dyes alone.

B. COLI: Gentian Violet Lactose Peptone Broth for the Detection of B. Coli in Milk, Kessler, M. A., and Swenarton, J. C. J. Bact. 14: No. 1, 47, 1927.

The medium is as follows:

Heat 1 liter of distilled water in a double boiler until water in outer vessel boils. Add 50 gm. of bile and 10 gm. of peptone, stirring until all ingredients are dissolved; continue boiling for one hour. Remove from flame and add 10 gm. of powdered lactose. Filter through cotton flannel until clear. To each liter of filtrate add gentian violet 1:25,000 adjusting the reaction of P_H 7.8 before adding 10 c.c. of a 1 per cent solution of brilliant green. Tube and autoclave for fifteen minutes at 15 pounds pressure.

Plain lactose broth, gentian violet lactose broth, and gentian violet lactose peptone bile have been compared as to their reliability for detecting B. coli in milk. It was found that gentian violet lactose peptone bile is the most reliable of these media for this purpose, and the formation of gas in this medium when inoculated with milk or diluted milk is a positive indication of the presence of B. coli which for practical purposes needs no confirmation.

TISSUE: A New Method For the Rapid Microscopic Diagnosis of Tumors, Dudgeon, L. S., and Patrick, C. V. Brit. J. Surg. 15: 250, October, 1927.

The tumor or other tissue is cut into, and the freshly cut surface scraped *firmly* with a sharp scalpel. The milky juice so obtained is spread evenly on slides and put immediately into Schaudinn's fluid *while still wet.* The composition of this fluid is as follows: (1) Saturated solution of mercuric chloride in distilled water, 2 parts; (2) Absolute alcohol, 1 part. A few drops of glacial acetic acid are added so as to obtain 4 per cent of the acid in the solution.

The wet films are allowed to remain in this fluid from two to ten minutes according to circumstances. If rapidity is essential, then an absolute minimum time of two minutes can be employed, but the best preparations are obtained by ten minutes' fixation. In some cases the wet films were placed in warm Schaudinn's fluid, but no advantage was obtained. Time will be saved if the tissue is scraped and the wet films are put into the fixative in the operating theater, so that fixation will take place during transit from the operating theater to the laboratory. The films are afterwards washed in spirit, and then in distilled water. Mayer's hemalum is employed as the nuclear stain, and eosin as the counterstain. Dehydration and clearing are done with absolute alcohol and zylol, and the films are then cover-slipped with Canada balsam. The total time required for the technical process can be as short as eight to ten minutes, but the time required for the miscroscopic examination is dependent upon the nature of the tissue and the rapidity of the investigator.

BACTERIOPHAGE: A Method of Isolation, De Groat, A. F. J. Immunol. **14**: No. 3, 175, 1927.

The organisms from 36 c.c. of a twenty-four-hour B. coli culture were concentrated to 4 c.c. To this were added 5 c.c. of a B. colibacteriophage filtrate and after shaking carefully the mixture was placed in the refrigerator three hours. The tube was then transferred to a bath at 60° C. for fifteen minutes, cooled, and centrifuged at high speed for twenty minutes. The supernatant fluid was discarded and the organisms were washed three times with cold, normal salt solution and suspended in 10 c.c. of distilled water. The latter operations were carried out as rapidly as possible. After twenty-four hours in the incubator at 37° C. the suspension was filtered through a fresh Berkefeld bougie and the filtrate preserved as "pure bacteriophage." Titrating this solution it was found to cause lysis in a dilution of $8\text{-}10$. The original filtrate produced lysis in a dilution of $9\text{-}10$.

Carrying this method out on a larger scale and washing the organisms only once with a large volume of saline, 50 c.c. quantities of "pure bacteriophage" solutions have been prepared which gave lysis in a dilution as high as $11\text{-}10$; original filtrate, $10\text{-}10$. This was done with the ordinary centrifuge of 300 c.c. capacity.

POLLEN EXTRACTS: The Preparation and Purification of Pollen Extracts, Alles, G. A., and Lawson, R. W. J. Immunol. **14**: No. 3, 181, 1927.

Two grams of dessicated pollen is extracted, with occasional shaking for twenty-four hours or longer, with 100 c.c. of the following glycerol extracting fluid: mix:

1 volume 0.30 M sodium dihydrogen phosphate (41.4 gm. NaH_2PO_4 12 H_2O per liter).

4 volumes 0.30 M disodium hydrogen phosphate (107.5 gm. Na_2HPO_4 12 H_2O per liter).

5 volumes c.p. glycerol.

The pollen extract is filtered through a Berkefeld filter. It is 1 to 50 with respect to extracted pollen and contains 20,000 pollen units per cubic centimeter. (The unit being defined as the active pollen substance extractable from 10-^6 gm. pollen, first defined by Noon for timothy pollen.) The extract contains 50 volumes per cent of glycerol, is isotonic in salt content, has an hydrogen-ion concentration within the limits for normal blood sera (P_H 7.4) and has the maximum buffering effect for this P_H that is obtainable with an isotonic salt content. Extracts made with this solvent and the similar one using mono- and dipotassium phosphates have retained satisfactory clinical activity for a period of two years and within the limits of error of skin testing methods appear to contain all of the salt and water soluble active substance present in pollen grain. The concentration of glycerol present is somewhat irritating on injection but not sufficiently so to be harmful. It is believed that glycerol is useful to insure stability, and practical sterility is secured by this concentration.

COLLOIDAL GOLD SOLUTION: A New Method of Preparation, Wuth, O., and Faupel, M. Bull. Johns Hopkins Hosp. 40: No. 5, 297, 1927.

The mode of procedure is as follows:

REAGENTS

1 per cent gold chloride (Merck).

3 per cent potassium carbonate.

2.5 per cent formaldehyde (prepared from Merck's 37 per cent formaldehyde 6.75 c.c. made up to 100 c.c.)

0.1 per cent tannic acid.

0.4 per cent phenol red (alcoholic).

Chemicals should be pure and glassware perfectly clean.

Preliminary titration: To 100 c.c. distilled water add 1 c.c. of gold chloride solution and 2 drops of phenol red indicator; then add potassium carbonate solution until a definite pink remains from one to one and one-half minutes (usually 0.5 c.c.).

Preparation of Colloidal Gold: Place 250 c.c. distilled water over bunsen flame; add 2.5 c.c. gold chloride and an amount of potassium carbonate calculated in the previous titration; after shaking, add 1 drop of 0.1 per cent tannic acid. Heat until solution boils vigorously. Remove flame. Add 1.5 c.c. formaldehyde quickly and shake continuously until solution turns a clear deep red. The temperature should be kept close to boiling point until the final color change has taken place. When the solution has cooled to room temperature, it should be tested with a known paretic and with a normal spinal fluid.

TISSUE TECHNIC: A Rapid Method for Zenker-Fixed Tissue, Hoag, F. H., and Jacobsen, V. C. Arch. Path. & Lab. Med. 4: No. 3, 397, 1927.

For fixation a piece 2 by 2 by 0.3 is placed in 80 to 100 c.c. of fixing fluid.

The following steps are then observed:

1. Fix in Zenker's fluid for three hours.
2. Wash three hours. (Usually removes all bichromate, but no harm is done if a trace remains, as it will be removed by washings of the section.)
3. Place in 80 per cent alcohol for one-half hour.
4. Place in 95 per cent alcohol for one-half hour.
5. Place in absolute alcohol, one-half hour.
6. Place in second absolute alcohol, one-half hour.
7. Chloroform, one-half hour.
8. Saturated solution of paraffin in chloroform, one-half hour.
9. Place in oven for ten minutes.
10. Embed at 53° C. for from twenty to thirty minutes.

Cutting and staining is done as follows:

1. Cut sections and mount on slide by albumin-glycerin method.
2. Place slide in an upright position in oven for twenty minutes.
3. Draw slide over edge of a hot plate several times to assure secure mounting.
4. Chill by holding slide on ice, and blot. (Steps 3 and 4 occupy five minutes.)
5. Place in xylol for five minutes.
6. Dip into absolute alcohol. Dip into 95 per cent alcohol. Dip into 80 per cent alcohol.
7. Place in 0.5 per cent iodine for five minutes.
8. Decolorize in four changes of 80 per cent alcohol.
9. Delafield's hematoxylin from ten to fifteen minutes.
10. Acid alcohol to remove any excess of hematoxylin. Wash in water. (Usually there is no excess of hematoxylin and this step is unnecessary.)
11. Restore hematoxylin blue with weak ammonia water. Wash in water.
12. Place in eosin or phloxine; then in absolute alcohol; then in xylol.
13. Mount in xylol balsam.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Diseases of the Skin**

A TEXTBOOK and reference manual on diseases of the skin written by a man eminent in his profession, and worthy of bearing his name. The illustrations are abundant and excellent. The text follows the general plan of the preceding edition. It is stimulating to find a textbook on dermatology in which the term eczema has been eliminated. "The term eczema means absolutely nothing. Practically all cases of eczema in the adult are examples of dermatophytosis of anaphylactic dermatitis, of dermatitis venenata."

In the preface the author anounces that the subject of anaphylactic dermatitis has received considerable mention. When we turn to the text however we find what the reviewer considers an altogether too brief discussion. We are still waiting for the textbook on dermatology which will present an adequate discussion of the relation of anaphylaxis or allergy to cutaneous manifestations.

Physical Diagnosis†

A COMPREHENSIVE textbook of diseases of the lungs, circulatory apparatus, and abdominal organs with brief additional chapters on regional diagnosis in the head, neck and extremities and on examination of the nervous system. This work is now in its fifth edition.

The Pneumothorax and Surgical Treatment of Pulmonary Tuberculosis‡

THIS volume is primarily a work on pneumothorax. In it are presented the history, mode of action, indications, counter indications, and technic of artificial pneumothorax and similar procedures together with a short chapter on thoracoplasty and other surgical measures.

Bacterial Vaccines and Their Position in Therapeutics§

RATHER a difficult subject to cover in eighty-three pages especially when one tries to mention all conditions in which vaccine therapy has been tried. The book gives a good bird's-eye view but at the end leaves you like the bird, up in the air. However, this weakness

*Diseases of the Skin. By Henry H. Hazen, A.M., M.D. Professor of Dermatology in the Medical Department of Georgetown University; Professor of Dermatology in the Medical Department of Howard University, Sometime Assistant in Dermatology in the Johns Hopkins University; Member of the American Dermatological Association. Third Edition. Two hundred forty-eight illustrations, including two color plates. Cloth. Pp. 571. The C. V. Mosby Company, St. Louis, Mo., 1927.

†Physical Diagnosis. By W. D. Rose, M.D., Associate Professor of Medicine in the University of Arkansas, Little Rock, Ark. Fifth Edition. Three Hundred Ten Illustrations and Three Color Plates. Cloth. Pp. 819. The C. V. Mosby Company, St. Louis, Mo., 1927.

‡The Pneumothorax and Surgical Treatment of Pulmonary Tuberculosis. By Clive Riviere, M.D. Lond., F.R.C.P., Physician, City of London Hospital for Diseases of the Heart and Lungs, Victoria Park, E.; Physician, E. London Hospital for Children, Shadwell, E. Second Edition. Illustrated. Cloth. Pp. 311. Oxford University Press. American Branch New York. 1927.

§Bacterial Vaccines and Their Position in Therapeutics. By Leonard S. Dudgeon, C.M.G., C.B.E., F.R.C.P. Lond., Professor of Pathology, University of London and Director of Pathology, St. Thomas' Hospital, Hon. Colonel, A.M.S. Cloth. Pp. 87. Price, $2.50. Paul B. Hoeber, N. Y., 1928.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

is inherent in the problem rather than in the author for six hundred or a thousand pages of utmost detail at the present time would in the end leave its reader in very much the same position. Of course, this does not apply to subjects such as typhoid or smallpox vaccination.

*International Clinics for December 1927 and March 1928**

WE CONGRATULATE the editors and publishers of International Clinics upon the completion of thirty-seven years of uninterrupted publication during which one hundred and fifty volumes have been issued.

The last volume for 1927 contains a collection of twenty-five selected clinical lectures delivered before the Interstate Post-Graduate Medical Association of North America during its European tour.

Both of these volumes follow the established procedure with a wide diversity of subjects and interests, with sections devoted to diagnosis and treatment, medicine, pediatrics, surgery, pathology, the medical specialties, medical history and a review of progress in medicine during 1927.

Diseases of the Mouth†

THE first one hundred and eighty-seven pages are devoted to maladies of the teeth and gums, the presentation being made with a view both to the local pathology and the remote systemic effects.

The remainder or greater portion of the book discusses most exhaustively diseases of the lips, the tongue, the throat, the salivary glands the maxillary sinus, the palate, the jaw bone, and the mouth as a whole (stomatitis). There is a chapter on specific infectious diseases, infections of the floor of the mouth and of the neck, one on diseases of the nerves, another on diseases of the blood and blood vessels in their relationship to local manifestations in the mouth, and additional chapters on fractures, tumors, cysts, and the relationship of oral sepsis to systemic disturbances. The volume is remarkably comprehensive and should serve as an excellent reference manual.

*International Clinics. Volume IV. Thirty-Seventh Series, 1927. Pp. 309. International Clinics, Volume I. Thirty-Eighth Series, 1928. Illustrated. Cloth. Pp. 307. J. B. Lippincott Company, Philadelphia, Pa.

†Diseases of the Mouth. By Sterling V. Mead, D.D.S., Professor of Oral Surgery and Diseases of the Mouth, Georgetown Dental School, with 274 original illustrations in the text and 31 full page color plates. Cloth. Pp. 578. The C. V. Mosby Company, St. Louis, Mo., 1928.

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EDITORIALS

Clinical Laboratory "Bunk"

IT IS a sad fact that as time has gone on and as clinical laboratory methods have been perfected, clinicians have seen in the improvements, opportunities for saving time by means of technicians. Many of them, the clinicians, not the technicians, think that in "the run of the mine" the laboratory will make a diagnosis in less time than they can, and so they make requests for "routine" or for "complete" tests. In not infrequent cases the clinician has a series of tests which he calls "routine" which may mean anything and which may include not only the usual qualitative urine and blood tests but also a whole series of quantitative studies embracing special blood counts and blood chemical estimations including the icterus index, cholesterol, calcium and carbon dioxide combining power. In some offices and in some institutions the laboratory studies precede the physical examination!

When such a situation exists there is good reason for *debunking,* to use

an ultramodern verb, for as every good clinical pathologist knows, most of the blood chemical laboratory work called for is *bunk*, to use the expressive noun.

The situation is that "the average physician in general practice will not see more than ten or a dozen cases a year in which a chemical analysis of the blood will be of any value to him in diagnosis or treatment."

Nowhere in recent literature has the state of affairs in respect to blood chemistry been exposed with greater clarity than in the article of Reed Rockwood on Chemical Tests of the Blood.

The commonest mistake, says Rockwood, is to order a "routine blood chemistry," a term which has no meaning. "Such a term really means that the clinician does not know anything of the indications for chemical analysis, and in a difficult case begins snatching at diagnostic straws. Such an order is a reflection on the diagnostic ability of the physician who gives it and does not come from those who are really acquainted with the practice of medicine." And if that is true of the doctor who gives his order *after* the physical examination, what could be said of the one who orders his laboratory work *before* he takes the history and makes the physical examination?

The remedy for this state of affairs appears to be to have the clinician order only the specific tests when they are indicated. But who shall decide when the indication has arisen? The general facts seem to be that the clinician does not know. But Rockwood says that the indications for the use of these tests are now just as definite as are those for certain other accessory procedures, and he goes on to show that the type of case in which abnormal conditions in the blood will occur can be predicted with a high degree of accuracy, and he also says "it is just as much the duty of the clinician to make this preliminary prediction as it is for him to make a preliminary diagnosis of pulmonary tuberculosis from the history and physical signs before ordering a roentgenogram."

It is of course one thing to criticize and another to suggest reform. Rockwood does both. It would be going too far afield to review his article *in extenso*. It is an article which should be read and digested by every clinician and by very many clinical pathologists. But this much we can say, that each blood chemical situation is discussed thoroughly enough and that from the discussions ten clinical aphorisms have been extracted. They might well occupy a prominent place in every medical office. They follow:

1. Never ask for both nonprotein nitrogen and urea in the same patient.

2. Except in emergency, never ask for a nonprotein nitrogen determination when the phenolsulphonephthalein secretion is normal. Determine the output of phenolsulphonephthalein first.

3. Never ask for the creatinine value of the blood unless the nonprotein nitrogen content is above 60 mg. per hundred cubic centimeters. Then determine the concentration of creatinine as a matter of routine.

4. Order determinations of the uric acid content only in cases of gout or suspected gout.

5. Order blood-sugar determinations only in cases of diabetes or suspected diabetes or hypoglycemia.

6. Ask for a test of the carbon dioxide combining power of plasma in:

a. Diabetic patients with diacetic acid in the urine.

b. Uremic patients with nitrogen retention and dyspnea.

c. Patients showing toxic symptoms who are receiving large doses of alkali.

d. Conditions associated with disturbed motility of the gastrointestinal tract with marked toxemia.

e. Tetany of any type.

7. Order chlorides, nonprotein nitrogen and carbon dioxide combining power determinations in all cases of disturbances of gastrointestinal motility with marked toxemia.

8. Ask for serum bilirubin or icterus index tests in cases of jaundice, but do not pay much attention to borderland values.

9. Ask for blood calcium determinations only in cases of tetany of unknown origin.

10. Order inorganic phosphorus tests, if practicable, only in cases of rickets and infantile tetany.

In connection with this work of Rockwood it is useful to consider an article by V. C. Myers. Rockwood writes from the standpoint of clinical medicine; Myers rather from that of the biochemist. One gains perspective from a perusal of both.

But also one gets from Myers' remarks an idea of how difficult it is apt to be to depend upon the work of mere technicians for accurate biochemic determinations. One is made to feel that we need more and better biochemists to oversee and evaluate the work of accurate technical assistants. So, says Myers, "although the help of technical assistants in a hospital laboratory is almost indispensable, such help is positively dangerous unless the work is under the direct supervision of some one with competent chemical training." Where does that put the blood chemical determinations of the ordinary back-room-doctor's-office technician? And beside, as Haldane says, "competent biochemists are very rare."

Well, what's the answer to it all? This, that the clinician should be thorough in his special work and that he should know the meaning of laboratory procedures. He should then ask his laboratory for reports on certain specific essential aspects of his cases, and not for unessential data. And he should assure himself that his highly specialized work in biochemistry is done by, or under the direct supervision of, competent persons.

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—*P. G. W.*

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News and Notes

The next annual meeting of the American Society of Clinical Pathologists will be held in Portland, Oregon, July 5, 6, and 8, 1929. All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west.

The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland, Oregon, one-half day will be devoted to a group presentation and general discussion of the subject of "Undulant Fever." Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee.

Dr. H. H. Foskett of Portland, Chairman of the Local Committee has been making extensive preparations for the meeting and assures the members good accommodations. Reservations, however, should be made now, specifying if these should be held over for the American Medical Association Convention. Those desiring same should communicate with the Secretary at once.

Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado.

The hospitals throughout the country are showing great interest in the work of the Registry of Technicians. Numerous requests for information have been received and also a large number of applications. The interest shown in this field of activity of the American Society of Clinical Pathologists evidences the existence of a problem which the organization has undertaken to solve.

REGISTRY OF TECHNICIANS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

In view of the present chaotic condition existing among the technical workers in the clinical laboratories of clinical pathologists and hospitals and the diversity of qualifications among these workers, the American Society of Clinical Pathologists, whose members are most vitally interested in the matter, has taken upon itself the task of clarifying the situation by instituting a Board of Registry and issuing certificates to properly qualified technicians. It is the endeavor of the Society to invest this useful calling with the dignity that it deserves and to create a proper spirit of appreciation for the members of a vocation which is dedicated to the aid of suffering humanity.

There is also an urgent need for proper control and survey of schools for laboratory technicians. It is the intention of the Registry to make periodical inspection of schools and laboratories that conduct courses of training and to give certificates only to such as meet the requirements. This move is necessitated by the existence of several schools of doubtful efficiency who by promises of lucrative positions will lure illy-prepared students to take brief courses of instruction at exorbitant rates. By the system of licensing the Society hopes to benefit prospective students in affording them opportunity to seek tuition in properly qualified schools or laboratories.

Another useful feature of the Registry is the Placement Bureau for Registered Technicians which promises to be a great boon, both to the laboratory as well as to the licensees.